1-1-2004

Immune modulation of antibody response to porcine reproductive and respiratory syndrome virus glycoprotein 5 (GP5) using anti-idiotypic antibodies in mouse model

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by

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A thesis submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Veterinary Preventive Medicine

Program of Study Committee:
En-Min Zhou, Major Professor
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Michael Wannemuehler

Iowa State University

Ames, Iowa

2004

Graduate College
Iowa State University

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has met the thesis requirements of Iowa State University

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Dedication

Sincere thanks to my wife, Fariba, who has patiently supported me in this endeavor.

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ABSTRACT

Porcine reproductive and respiratory syndrome (PRRS), caused by the PRRS virus (PRRSV), is a disease of great economic importance for the swine industry worldwide. PRRSV is a member of the family *Arteriviridae* in the order *Nidovirales*. PRRSV is an enveloped positive single-stranded RNA virus, which encodes an approximately 4,000-aminoacid large replicase polyprotein (open reading frame [ORF] 1a and 1b) and six structural proteins of 130 to 265 amino acids (ORFs 2 to 7). Three of these structural proteins have been identified as envelope (25 kDa), matrix (19 kDa), and nucleocapsid (15 kDa) proteins, i.e. GP5, M, and N proteins, respectively. PRRSV infection poses a challenge to current serodiagnostic and vaccination strategies. A better understanding of the mechanisms that regulate immunity to this virus will aid in the development of more effective vaccines, as well as serodiagnostic assays.

The idiotype (Id) network theory of immune regulation offered by Jerne (1974), proposing that the immune response to a given antigen can be regulated by a series of Id and its serological counterpart, an anti-idiotypic antibody (anti-Id or Ab2), has been found useful for the production of diagnostic reagents and vaccines. Upon immunization with an idiotypic antibody (Ab1), the corresponding anti-Ids are elicited and can be serologically classified into four categories (Ab2α, Ab2β, Ab2γ and Ab2ε). Ab2β, an internal image anti-Id Ab2, recognizes an Id within the antigen-combining site and bears a structural resemblance to the original antigen. The concept of internal image stems from the possibility of raising Ab3 antibodies that would recognize the original Ag. If one takes into account both the specificity and the idiotypic characteristics of Ab1 molecules, four discrete types of Ab3 molecules may

be anticipated: Id^-Ag^-, Id^+Ag^-, Id^-Ag^+ and Id^+Ag^+, the later being very similar to Ab1 and termed Ab1'. We have previously suggested the possible role of auto-anti-idiotypic antibodies (AAb2) in immunity to PRRSV infection and characterized pig AAb2 specific for PRRSV glycoprotein (GP5) as an internal image anti-Id (Ab2β). To further confirm the role of Id-anti-Id network in PRRS infection, two studies were performed in a mouse model. In the first study, a monoclonal anti-idiotypic antibody (anti-Id), designated as M8G, was generated against a monoclonal antibody (MAb 25C) specific for PRRSV glycoprotein (GP5). Observations that (i) the anti-Id inhibited the binding of MAb 25C to PRRSV antigen and (ii) the Id-anti-Id interaction could be inhibited by PRRSV antigen indicated that the Id was located within or near the antigen combining site. The anti-anti-Id antibodies (Ab3) induced with anti-Id MAb2, competed with MAb1 binding to PRRSV antigen. Furthermore, the binding of anti-anti-Id to MAb2 was inhibited with PRRSV antigen, indicating that anti-anti-Id mimics the structure of the epitope in GP5 which was recognized with Ab1. These serologic findings suggest that anti-Id MAb2 (M8G) carries the internal image of the antigen. In the second study, BALB/c mice were immunized with AAb2 and the immune sera (Ab3) were tested for the presence of anti-GP5 antibodies and the expression of the Id of Ab1 specific for GP5 of PRRSV. Mice immunized with AAb2 were able to produce Ab1-like antibody (Id^+Ag^+) responses, i.e., they recognized the same or a similar epitope as Ab1 and possessed the Ab1 Id, without subsequent exposure to the original antigen. This was demonstrated by the following results (i) inhibition of the binding of mouse immune serum to AAb2 by PRRSV antigen, (ii) inhibition of binding of mice immune serum to PRRSV antigen by pig antisera, (iii) inhibition of AAb2 the binding to MAb1-25C by Ab3, and (iv) inhibition of binding of mouse immune serum to AAb2 by pig antisera. We conclude that pig

auto-anti-Id serologically mimics an Ab1-defined GP5 epitope sufficiently to function as a surrogate antigen for inducing anti-PRRS virus GP5 responses.

These results indicate that Id-anti-Id network may be operational in modulating the immune response to GP5 in PRRSV infection.

CHAPTER 1. GENERAL INTRODUCTION

Thesis Organization

This thesis consists of four chapters. Chapter 1 is a general introduction and review of the literature on porcine reproductive and respiratory syndrome (PRRS) and idiotypes and anti-idiotypic antibodies. Chapter 2 and 3 consist of two papers. The first paper, "Monoclonal anti-idiotypic antibody specific for GP5 antigen of porcine reproductive and respiratory syndrome virus elicits specific immune response in mice" is to be submitted for publication in Viral Immunology. The second paper, "Swine auto-anti-idiotypic antibody specific for antibodies against GP5 antigen of porcine reproductive and respiratory syndrome virus elicits anti-GP5 antibody response in mice" will be submitted to the Journal of Immunology. The last chapter contains the general conclusions of the research studies. References for the general introduction and the literature review are in alphabetical order and follow the literature review. References for each paper follow the discussion section of each paper while the general discussion and conclusion references follow that section.

Review of Literature

Part I: Porcine Reproductive and Respiratory Syndrome

History of PRRS

Porcine reproductive and respiratory syndrome (PRRS) was first described in the United States in 1987 as a new viral disease of swine (Hill, 1990) and appeared in Europe in 1990 (Terpstra et al., 1991). The syndrome was initially called mystery swine disease (MSD) (Wensvoort et al., 1991) in the United States since no known swine pathogens could be implicated. A number of titles have been used to describe this disease syndrome: swine infertility and respiratory syndrome (SIRS) (Benfield et al., 1992; Collins et al., 1992; Morrison et al., 1992; Yoon et al., 1992), porcine epidemic abortions and respiratory syndrome (PEARS) (Pol et al., 1991; Terpstra et al., 1991), blue-eared pig disease (White, 1991) as well as others. Since the First International Symposium on SIRS/PRRS (1992), PRRS has been the internationally recognized name applied to the syndrome (Meredith, 1993).

Porcine reproductive and respiratory syndrome virus (PRRSV)

Porcine reproductive and respiratory syndrome virus (PRRSV) is a recently emerged pathogen of domesticated swine. PRRSV is a small, enveloped, positive sense single-stranded RNA virus that causes reproductive failure in breeding swine and respiratory problems in young pigs. PRRSV is a member of the family *Arteriviridae* in the order *Nidovirales* (Cavanagh, 1997). The *arterivirus* family consists of PRRSV, lactate

dehydrogenase-elevating virus (LDV), equine arteritis virus (EAV), and simian hemorrhagic fever virus (SHFV) (Plagemann, 1992). The 5'-capped (Sagripanti et al., 1986) and 3'-polyadenylated (Brinton et al., 1986; Sagripanti, 1985; van Berlo et al., 1982) RNA is polycistronic, containing (5' to 3') two large replicase open reading frames (ORFs), 1a and 1b, and several smaller ORFs (Conzelmann et al., 1993; Kuo et al., 1992; Murtaugh et al., 1995; Smith et al., 1997). In the infected cell, arteriviruses produce a nested set of six to eight major coterminal subgenomic mRNAs (sgmRNAs) each thought to express only the relevant 5'-terminal ORF. These sgmRNAs have a leader sequence derived from the 5' end of the genome that is joined at specific leader-body junction sites located downstream by an unclear discontinuous transcription mechanism (Lai, 1995). The sgmRNAs of PRRSV encode four glycoproteins (GP2 to 5, encoded by sgmRNAs 2 to 5), an unglycosylated membrane protein (M, encoded by sgmRNA 6), and a nucleocapsid protein (N, encoded by sgmRNA 7) (Bautista et al., 1996; Mardassi et al., 1996; Meng et al., 1994; Meulenberg et al., 1993; Mounir et al., 1995; Nelson et al. 1995). The European prototype strain of PRRSV, Lelystad Virus (LV), contains all six of these proteins in the viral genom (Meulenberg et al., 1995; Meulenberg et al., 1996; van Nieuwstadt et al., 1996), but only the proteins encoded by ORFs 5 to 7 have conclusively been demonstrated to be in the virion of North American isolates (Bautista et al., 1996; Nelson et al., 1995; Pirzadeh and Dea, 1997). Nucleotide and amino acid sequence comparisons of the 3'-terminal ORFs 2 to 7 have shown that there are significant differences between PRRSV strains native to Europe and those found in North America (Kapur et al., 1996; Murtaugh et al., 1995). Therefore, although these two PRRSV strains cause similar diseases (Benfield et al., 1992; Wensvoort et al., 1991), they are genotypically different in the genes encoding structural proteins.

Clinical Manifestations

Direct contact is the most prominent route of exposure to PRRSV. Persistence of virus in the respiratory tract and lymphoid tissues, predominantly tonsil, may occur for up to 157 days postinfection (Wills et al., 1997). Production losses and clinical signs will vary considerably between farms, depending on the strain of virus (Halbur et al., 1996), management practices (Wensvoort, 1993), age of the pig and the herd immune status (Keffaber, 1989; Wensvoort, 1993). The wide range of variation in the severity of PRRSV infection which is commonly observed in both breeding animals and in young pigs may be influenced by coinfection with other swine pathogens such as *Salmonella choleraesuis*, *Streptococcus suis*, *Haemophilus parasuis*, *Actinobacillus pleuropneumoniae*, swine influenza virus, porcine respiratory coronavirus, pseudorabies virus, or mycoplasmas (Galaina et al., 1994; Thacker et al., 1999). Variation in severity may also be due to strain differences among PRRSV (Epperson and Holler, 1997; Halbur and Bush, 1997; Hurd et al., 1998; Joo et al., 1998a; Mengeling et al., 1997; Rossow, 1997; Zimmerman et al., 1997). Low-virulence strains can cause subclinical epidemic or endemic infections of herds (Morrison et al., 1992), whereas highly virulent strains can cause severe clinical illness. Disease in a naive herd exposed to a virulent strain can be devastating. Clinical signs may include anorexia, fever, and lethargy in sows or gilts for one to seven days. Cyanotic (blue) ears, vulva, tails, abdomens, and snouts are most often seen with infection caused by European strains. Reproductive failure is characterized by late-term abortions, increased numbers of stillborn fetuses, and/or premature weak pigs. Infection during mid-gestation may be followed by abortions, mummified fetuses, early embryonic death, and infertility. In some herds, there is a 1-4% mortality in acutely ill sows (Loula,

1991) that is sometimes associated with lesions of pulmonary edema or cystitis/nephritis (Hopper et al., 1992). Acute phases of disease typically last two to three months after which reproductive parameters often return to normal. A persistent reproductive form exhibits decreases in the farrowing rate and irregular returns to estrus. The enzootically infected breeding herd may appear normal on clinical evaluation. Cycles of high conception failure (15-20 percent), stillbirths (10-15 percent), or preweaning mortality (15-20 percent) are common. Chronic PRRS problems are typical in seropositive herds which add seronegative, susceptible animals without proper isolation and acclimatization.

Piglets can be infected in utero and be born weak. This is often associated with premature farrowing. Infected litters are weak, unthrifty, or splay-legged. Signs in congenitally infected pigs may manifest as muscle tremors, eyelid edema, conjunctivitis and/or fever. Pigs may display respiratory dyspnea ("thumping"). Increasing incidence of bruising and hemorrhaging during processing has been reported. Preweaning mortality has been reported to be as high as 60 percent (Benfield et al., 1999). Clinical signs in 3 to 10 week old pigs are quite variable. Many seropositive herds have no clinical signs of PRRS in growing pigs, particularly when weaned early. Most consistent clinical signs observed are associated with respiratory disease or ill-thrift. Boars may show anorexia, lethargy, respiratory clinical signs, and also lack libido and have variable reduction in semen quality (Feitsma et al., 1992).

The clinical signs of endemic PRRSV infection in herds are most often obvious in weaned pigs in the nurseries or early in the finishing barns when a limited outbreak occurs in a susceptible population of pigs (Stevenson et al., 1993). Clinical signs in the breeding herd in endemically infected herds are usually limited to susceptible gilts or replacement boars that

are exposed to PRRSV after introduction into the herd (Dee and Joo, 1994b; Dee et al., 1996).

Immunity to PRRS

Exposure of pigs to PRRSV induces immunity that may protect against reexposure to the same virus. However, pigs exposed to PRRSV also demonstrate prolonged viremia and persistent infection, they may continue to shed virus, they can become reinfected, and they may suffer a repeat episode of the disease. These observations indicate the existence of a complex immunological interaction between PRRSV and pigs that involves both induction and subversion of host defenses.

Innate response to PRRSV infection

Immunity to PRRSV begins with an innate antiviral response in the cytoplasm of an infected macrophage. Initial resistance to PRRSV is dependent on antiviral defenses of the infected cell and of the innate immune system in the week or more prior to the development of adaptive immunity. In an infected pig, the first sign of danger occurs in the cytoplasm of an infected macrophage. In viral infections, the presence of double-stranded RNA triggers a variety of antiviral functions, of which the induction of the type I interferons, interferon α and β (IFN α/β), is a hallmark of cellular antiviral defense (Pfeffer et al., 1998; Tizard, 1995; Vicek and Sen, 1996). Although PRRSV has positive-sense, single-stranded RNA molecules, and PRRSV mRNA appears to have a high degree of double-stranded structure, there is no evidence of accelerated RNA degradation or reduced levels of protein synthesis in PRRSV-infected macrophages. This indirect evidence for a lack of intracellular antiviral activity is

consistent with observations that PRRSV infection does not elicit type 1 interferon expression in vitro or in vivo (Albina et al., 1998a; Buddaert et al., 1998; van Reeth et al., 1999). Absence of IFNα production is dependent on replication by PRRSV. The down-regulation of IFNα production facilitates PRRSV replication since elevation of type I interferons by in vivo stimulation or exogenous administration substantially reduces viral growth and enhances humoral immune responses (Albina et al., 1998a; Le Bon et al., 2001).

In addition to IFNα production, inflammatory cytokine expression also is important in the initial response to a variety of viral respiratory infections (van Reeth and Nauwynck, 2000). PRRSV infection failed to elicit any significant cytokine expression (Thanawongnuwech et al., 2001; van Reeth et al., 1999). TNF-α expression was suppressed or declined markedly after PRRSV infection (Chiou et al., 2000; Lopez-Fuertes et al., 2000). In virus infection, TNF-α and IL-1β are important activators of the nuclear transcription factor, NF-κB (Christman et al., 1998; Christman et al., 2000; DiDonato et al., 1997; Regnier et al., 1997). NF-κB plays a central role in the innate response to infection by regulating the transcription of more than 100 genes, including inflammatory and immunoregulatory cytokines, antigen receptors, adhesion molecules, inhibitors of apoptosis, acute phase proteins, and innate effector molecules (Schmid and Adler, 2000). It has been speculated that the mild or subclinical respiratory involvement in PRRSV infection may be due to the lack of activation of NF-κB. Moreover, the lack of an acute inflammatory response and weak innate antiviral activity may also result in incomplete stimulation of antigen-specific immune responses, and allow for the establishment of persistent infection (Murtaugh et al., 2002). The experimental results and observations to date suggest that the initial, innate immune response to PRRSV is

weak. In contrast to many host-virus interactions, the IFNα response at the site of infection is nil, the production of inflammatory cytokines is minimal and delayed, and the recruitment and activation of NK cells is unremarkable. The lack of an IFNα response is important, because IFNα -mediated events inhibit PRRSV replication in vitro (Albina et al., 1998a; Buddaert et al., 1998) and because elevation of IFNα in vivo by preinfection with PRCV substantially attenuates subsequent PRRSV replication (Buddaert et al., 1998). Moreover, a weak innate immune response to PRRSV is consistent with a suboptimal stimulation of antigen-specific humoral and cell-mediated immunity. Influenza virus elicits potent inflammatory cytokine and interferon responses in the lung, and is rapidly cleared within 1 week of infection (Brown et al., 1993), whereas PRRSV viremia has been observed for 6 to 7 weeks after infection (Suarez, 2000), and the virus can be reisolated months after initial infection (Albina et al., 1994; Christianson et al., 1992; Labarque et al., 2000; Rossow, 1998).

Humoral immune response to PRRSV

The antibody response to PRRSV has been studied extensively. Anti-PRRSV IgM antibodies appear in serum by 5 to 7 days postinoculation (PI) and then decline rapidly to undetectable levels after 2 to 3 weeks (Joo et al., 1997; Loemba et al., 1996; Park et al., 1995; Yoon et al., 1995). Anti-PRRSV IgG antibodies are first detected 7 to 10 days PI (Labarque et al., 2000; Loemba et al., 1996; Yoon et al., 1995), peak at 2 to 4 weeks PI, remain constant for a period of months, and then decline to low levels by 300 days PI (Nelson et al., 1994; Nielsen and Bøtner, 1997). Anti-PRRSV antibodies may persist in blood for the lifetime of commercial pigs. A group of 11 pigs infected experimentally remained seropositive for 600 days and

were protected completely against challenge with the homologous strain of virus (Lager et al., 1997).

Immunoglobulins of the IgG1 subclass appear at day 9 PI, while IgG2 subclass antibodies are first detected at day 14 PI, but at a lower level (Labarque et al., 2000). Anti-PRRSV IgA can be detected in serum at 14 days PI, reaches a maximum at 25 days PI, and remains detectable until 35 days PI. The kinetics of anti-PRRSV antibody isotypes in bronchoalveolar lavage (BAL) fluid are similar to those in serum, indicating these antibodies extravasate from the vasculature (Labarque et al., 2000). The antibodies in BAL may contribute to the clearance of PRRSV from the lung, but are unable to completely eliminate the virus (Labarque et al., 2000). Serum neutralizing (SN) antibodies are reported to appear at about 3 weeks post infection (Albina et al., 1998b; Eichhorn and Frost et al., 1997; Loemba et al., 1996), although SN antibodies have been reported at 9 days PI (Yoon et al., 1994). Slow-reacting complement-requiring neutralizing antibodies have been demonstrated as early as 8 days PI (Jusa et al., 1996; Takikawa et al., 1997). SN antibodies are maintained for long periods, but at low levels (Albina et al., 1998b; Labarque et al., 2000; Loemba et al., 1996; Yoon et al., 1995). Substantial variation has been noted in the SN antibody responses of individual pigs, in both the kinetics of appearance and in titer values (Loemba et al., 1996; Yoon et al., 1995). Nelson et al. (1994) also reported animals that failed to seroconvert in the SN test. Anti-PRRSV immunoglobulins in serum after PRRSV infection are directed primarily against PRRSV N protein (encoded by ORF 7) and M protein (encoded by ORF 6) (Dea et al., 2000). Antibodies against N protein can be detected within 7 days of infection, whereas antibodies against M protein appear by the end of the second week PI (Loemba et al., 1996; Nelson et

al., 1994; Yoon et al., 1995). Anti-GP5 (encoded by ORF 5) antibodies may also appear by day 7 PI (Dea et al., 2000; Loemba et al., 1996; Nelson et al., 1994; Yoon et al., 1995). Neutralizing antibodies to PRRSV have been reported with specificities against GP5, GP4, and M (Halbur et al., 1997; Meulenberg et al., 1997; Ostrowski et al., 2002; Pirzadeh and Dea, 1997; Pirzadeh and Dea, 1998; Weiland et al., 1999; Yang et al., 2000). Murine monoclonal antibodies against GP5 are more effective in virus neutralization than monoclonal antibodies to GP4 (Weiland et al., 1999). The immune response to individual structural proteins in serum of pigs recovered from PRRSV infection has been examined by serological reactivity to GP3, GP4, GP5, and N expressed as recombinant fusion proteins with glutathione-S-transferase (GST). SN titers were significantly correlated with anti-GP5 titers but not with anti-GP4 titers (Gonin et al., 1999; Ostrowski et al., 2002; Pirzadeh and Dea, 1997; Weiland et al., 1999). Antibodies against GP3 also may have a role in protection against PRRSV infection, though the data are less direct (Dea et al., 2000; Plana Duran et al., 1997).

Antibody responses are elicited to nonstructural proteins (nsp) of the replicase complex, particularly the nsp 2 polypeptide encoding a putative cysteine protease (Meulenberg et al., 1993; Oleksiewicz et al., 2001b). The antibodies recognize linear epitopes in nsp 2 and appear within 1 to 4 weeks of infection (Oleksiewicz et al., 2001b). These antibodies are detected in boar semen and their presence is correlated with the duration of PRRSV excretion in semen (Oleksiewicz et al., 2001a). Humoral immune responses have also been evaluated in response to DNA vaccination with plasmids containing expression cassettes for various structural proteins. Antibodies against GP5 were detected 15 days after ORF 5 DNA

vaccination by indirect immunofluorescence (IFA) and ELISA, and neutralizing antibodies were demonstrated 2 to 3 weeks after the second booster injection, 8 to 9 weeks after the first inoculation (Pirzadeh and Dea, 1998). After PRRSV challenge, the neutralizing titers rose to approximately 128. In another DNA vaccination study, ELISA antibodies were detected against N after three immunizations and against M after four immunizations (Kwang et al., 1999).

Colostral antibody responses are closely correlated with serum antibody responses. For example, sera-positive sows were colostrum-positive, and all colostrum-negative sows also were sera-negative (Eichhorn and Frost, 1997). Because the concentration of IgG in colostrum exceeds that found in serum (Pastoret et al., 1998), sows may be colostrum-positive but sera-negative (Eichhorn and Frost, 1997). Colostral IgG is absorbed by suckling newborn piglets within 24 to 48 h of birth via the apical tubulovesicular membrane system (ATVM) into serum (Pastoret et al., 1998). These maternal antibodies are reported to persist in piglets up to 6 to 8 weeks of age (Chung et al., 1997; Houben et al., 1995).

Attenuated, live vaccines have been widely used in swine farming and have proven effective in reducing disease severity, duration of viremia, virus shedding, and the frequency of PRRSV infection (Christopher-Hennings et al., 1997; Dee et al., 1998; Mavromatis et al., 1999; Mengeling et al., 1999; Nielsen et al., 1997; van Woensel et al., 1998). The humoral immune response is presumed to play an important role in resistance to reinfection and in prevention or reduction of viral spread from animal to animal, since neutralizing antibodies have the potential to clear free virus from the circulation (Albina et al., 1994; Molitor et al., 1997; Ostrowski et al., 2002; Yoon et al., 1995). Reproductive failure due to PRRSV

infection also has been prevented by administration of serum containing a high titer of SN antibody (Osorio et al., 2002).

Despite these results, the role of neutralizing antibodies in prevention of disease and in protection of pigs from infection under field conditions is not clear. Since viremia may occur in the presence of SN antibodies (Christianson et al., 1992; Dee et al., 1998; Rossow et al., 1994; Wills et al., 1997), the level of neutralizing antibodies normally generated against PRRSV may not be sufficient to control the replication of the virus. Selection of neutralization escape mutants has been proposed as one mechanism to account for inefficient neutralization (Bautista and Molitor, 1997; Rowland et al., 1999), but neutralization escape mutants or genetic variation in the neutralization-sensitive ectodomain regions of GP5 have not been observed in experimental or field settings (Chang et al., 2002; Le Gall et al., 1997; Madsen et al., 1998; Rowland et al., 1999; Storgaard et al., 1999). Moreover, all monoclonal antibodies bind both glycosylated and nonglycosylated forms of GP5, indicating that glycosylation is not associated with neutralizing epitopes of the protein (Dea et al., 2000; Pirzadeh and Dea, 1997; Pirzadeh and Dea, 1998; Weiland et al., 1999; Zhang et al., 1999).

The phenomenon of antibody-dependent enhancement (ADE) also might reduce the effectiveness of a neutralizing antibody response (Kurane et al., 1991). Low titers of neutralizing antibodies may increase the association of viral particles with permissive macrophages through binding of virus-antibody complexes to the Fc receptor, and thus facilitate viral binding and uptake through the macrophage PRRSV binding protein (Choi et al., 1992; Christianson et al., 1993; Yoon et al., 1996; Yoon et al., 1997). Infection of alveolar macrophages by PRRSV is significantly enhanced in vitro in the presence of diluted

anti-PRRSV antisera (Yoon et al., 1996), and the mean level and duration of viremia are greater in pigs injected with SN antibodies prior to virus challenge than in pigs injected with normal IgG (Christianson et al., 1993; Yoon et al., 1996). The prolonged duration of viremia and virus isolation from the tissues in piglets with low maternal antibodies also suggests ADE of PRRS (Shibata et al., 1998). Alternatively, slow development of an effective neutralizing antibody response might be due to the presence of immunodominant nonneutralizing epitopes on GP5 that act as a decoy to delay the induction of antibody production directed against neutralizing epitopes of the protein (Ostrowski et al., 2002).

However, the appearance of antibodies in serum to an immunodominant, highly conserved, nonneutralizing GP5 epitope is itself slow and incomplete, with only 50% of animals seroconverting 56 days after PRRS virus infection (Oleksiewicz et al., 2002; Rodriguez et al., 2001). Whether induction of antibody production to neutralizing epitopes is an inherent characteristic of the epitopes themselves or is determined by a decoy process is not known at this time.

Cell-mediated immune response to PRRSV

PRRSV infection in the lung peaks at approximately day 9 after infection and foci of infection decline to near zero by day 20, although virus still may be isolated from lung fluids for extended periods (Labarque et al., 2000; Mengeling et al., 1996; Samsom et al., 2000). During and subsequent to the decrease in PRRSV titer, lymphocyte numbers may either remain low or constant (Labarque et al., 2000) or increase substantially (Samsom et al., 2000) in the lung. Samson et al. (2000) identified a CD4(-) CD8(+) T cell population that

increased in the lung at later times. The lymphocytes were classified as cytotoxic T cells based on a cell marker phenotype of CD6(-) CD8(+). Shimizu et al. (1996) also observed a substantial increase in CD8(+) T cells that peaked at 25–35 days after infection. In peripheral blood during the first week of PRRSV infection, there is a transient leukopenia and lymphopenia in peripheral blood that resolves in 8 to10 days (Christianson et al., 1993; Nielsen and Bøtner, 1997). The absence of a corresponding increase in lymphocyte numbers in the bronchoalveolar fluids at this time indicates that this change is not a direct response to the virus (Labarque et al., 2000; Samsom et al., 2000).

PRRSV-specific T cell responses first appear in peripheral blood at approximately 4 weeks after infection (Bautista and Molitor, 1997). An antigen-specific proliferative response was observed for 5 weeks, that is, until 9 weeks after infection. The proliferative response was inhibited approximately 80% and 90% by anti-CD4 and anti-MHC II antibodies, respectively, and by 50% and 75% by anti-CD8 and anti-MHC I, respectively (Bautista and Molitor, 1997). Challenge with the homologous virus restimulated a specific proliferative response in peripheral blood mononuclear cells, which was detected for 3 weeks (Bautista and Molitor, 1997). In vivo specific cell-mediated reactivity to PRRSV antigen was confirmed by a delayed type hypersensitivity reaction to inactivated PRRSV (Bautista and Molitor, 1997). Lopez Fuertes et al. (1999) also reported PRRSV-specific lymphocyte proliferation at 4 weeks PI. In this case, the responsive T cell type also was predominantly CD4(+) and was present for a period of 10 weeks after infection. Stimulated cells expressed IFNγ and IL-2, but not IL-4 or IL-10, suggesting that the CD4(+) T cells possessed a type I cytokine expression phenotype characteristic of cell-mediated immune responses to

intracellular pathogens (Lopez Fuertes et al., 1999). In vitro restimulation of PBMC with PRRSV antigen and IL-2 showed that the responding cells were primarily CD8(+) (Zuckermann and Husmann, 1996). T-cell proliferation is induced in response to individually purified structural proteins GP5, M, and N (Bautista et al., 1999). Expression of GP2, GP4, GP5, M, and N individually in a vaccinia virus system revealed that the nonglycosylated M protein was the most potent inducer of proliferation, followed by GP5, GP3, and GP2, whereas N was the weakest inducer (Bautista et al., 1999). Host IFNγ production is central to protective mechanisms in a variety of cytopathic viral infections in murine models (Ramsay et al., 1993; Zinkernagel et al., 1996). Thus, it is possible that IFNγ also mediates protection against viral infections in pigs. Support for this notion exists in the observation that the efficacy of pseudorabies virus vaccines is positively correlated with the generation of virus-specific IFNγ-producing cells, but not with the generation of neutralizing antibodies (Zuckermann et al., 1998; Zuckermann et al., 1999). IFNγ mRNA has been detected in the lymph nodes, lungs and peripheral blood mononuclear cells of PRRSV-infected pigs (Lopez Fuertes et al., 1999; Rowland et al., 2001). Moreover, IFNγ blocks PRRS virus replication in cultured cells (Bautista et al., 1999) apparently by the inhibition of viral RNA synthesis via a double-stranded RNA-inducible protein kinase (Rowland et al., 2001). The observation that PRRSV persists in lung and lymph nodes despite the presence of neutralizing antibodies in serum and bronchoalveolar lavage fluid (Albina et al., 1994; Chung et al., 1997; Labarque et al., 2000; Wills et al., 1997; Zimmerman, 1999) argues that cell-mediated immunity is necessary for the complete elimination of the virus. Nevertheless, the existence of PRRSV persistence also suggests that cell-mediated immunity is not potent and that IFNγ production is either weak or ineffective.

Analyses of the IFNγ response to PRRSV infection revealed that the frequency of virus-specific IFNγ-producing lymphocytes in the blood of pigs ranged from approximately 50 to $100/10^6$ PBMC at 13 weeks after infection with virulent PRRSV or 8 weeks after a live, attenuated booster vaccination (Meier et al., 2000). In contrast, greater than 400 virus-specific IFNγ-producing lymphocytes/10^6 PBMC were detected within 2 weeks after secondary immunization with a live, attenuated pseudorabies vaccine (Meier et al., 2000). This level of IFNγ response was not achieved until 23 weeks after a similar immunization protocol with a PRRSV vaccine. Thus, the cell-mediated immune response to PRRSV, as measured by the frequency of PRRS virus-specific IFNγ secreting cells, is weak. The significance of a relatively low IFNγ response as it relates to protective immunity is not known. In pseudorabies virus, a frequency of >400 antigen-specific IFNγ secreting cells/10^6 PBMC correlates with effective protection of pigs against the clinical consequences of challenge with a virulent form of this virus (Zuckermann et al., 1999). Pigs that have recovered from a previous PRRSV infection demonstrate resistance to a secondary challenge by the homologous strain at least up to 604 days after the initial infection (Lager et al., 1997). After re-challange, there is a high frequency of PRRS virus-specific IFNγ secreting cells (450 ± $50/10^6$ PBMC), whereas the humoral immune response is barely detectable (Meier et al., 2000). The intensity of the IFNγ response to either wild type or attenuated PRRSV increases gradually over a period of months, while humoral immunity decreases (Meier et al., 2000). These contrasting changes in immune response are intriguing, but the mechanisms responsible have not been identified. Because IFNγ plays a key role in cell-mediated immune responses, factors that increase its expression might enhance anti-PRRSV cell-mediated responses. IL-12 and IFNα, in particular, are involved in the differentiation of naive T cells

into antigen-specific IFNγ-secreting cells (Banyer et al. 2000; Cousens et al. 1999; Kadowaki et al. 2000; Tough et al. 1999). In pigs, potently immunogenic viruses induce high levels of inflammatory cytokine and type 1 interferon expression (van Reeth et al. 1999), whereas PRRSV does not stimulate significant amounts of either in the lung (Albina et al. 1998a; Buddaert et al., 1998; van Reeth et al. 1999). It is possible that the production of type 1 interferon or appropriately stimulatory cytokines during the innate immune response are a critical element that is missing from the anti-PRRSV response (Murtaugh et al., 2002). Novel vaccine approaches against PRRSV have been used for further characterization of cell-mediated immune responses and for evaluation of protective efficacy. DNA immunization of pigs with plasmids encoding PRRSV GP4, GP5, M, or N induced the appearance of peripheral blood lymphocytes responsive to PRRSV measurable by antigen-specific proliferation or IFN production (Kwang et al., 1999). The responses were observed after two immunizations, at least 3 weeks earlier than ELISA or Western blot evidence of an immune response was detected (Kwang et al., 1999). GP5-specific lymphocyte proliferation was also observed in pigs immunized with plasmid DNA encoding ORF 5 (Kwang et al., 1999). In this case, specific neutralizing antibodies also were elicited, and pigs were protected from viremia and macroscopic lung lesions induced in unvaccinated pigs by virulent PRRSV (Pirzadeh and Dea, 1998). Plana Duran et al. (1997) also reported that immunization with GP3 or GP5 protein confers protection against challenge with virulent PRRSV, though immunization with GP5 did not protect pigs from challenge in another study (Pirzadeh and Dea, 1998).

Prevention and Control

Prevention of PRRS in a naive herd depends on the ability to prevent introduction of infected animals. This is a challenge considering the number of routes of transmission possible. Primary transmission is via direct contact but aerosol and fomite transmission are possible. Pigs can be nonclinical carriers for several months and PRRSV can persist in some animals despite high levels of antiviral antibodies; in boars, this is associated with long-term, intermittent seminal excretion of the virus (Christopher-Hennings et al., 1995; Gradil et al., 1996). Therefore, it is critical to routinely isolate and test breeding stock before introducing them to a PPRS-negative herd. Replacement stock to be added to naïve herds should be obtained from known negative sources that carry out a regular schedule of herd monitoring (Benfield et al., 1999). Following the arrival of new stock, all animals should be blood-tested 14 days following entry into the isolation building. Isolation periods should be at least 30 days to allow sufficient time to obtain laboratory results prior to animal introduction (Benfield et al., 1999). Serological tests cannot discriminate between seropositive animals which have cleared PRRSV infection and carrier animals. Since there are multiple strains of the PRRSV, serological tests must be correctly selected. Control strategies must be tailored to individual farms. Strategies must be based on epidemiology of the virus, facilities available, pathogenicity of virus strain involved, and management resources.

PRRSV infection poses a challenge to current vaccination strategies. Several studies have stablished that vaccination against PRRS can result in protective immunity (Gorcyca et al., 1995; Gorcyca et al., 1996b; Hesse et al., 1996a, b; Mengeling et al., 1996;). Although live PRRSV vaccines provide protection against homologous challenge, the genetic diversity of

field PRRSV isolates is very high, and vaccine effect against heterologous challenge may be limited (van Woensel et al., 1998). Also, live PRRSV vaccines have been observed to revert to virulence (Bøtner et al., 1997; Storgaard et al., 1999), and the safer, killed vaccines have so far proved less effective (Nielsen et al., 1997). Addressing these problems might involve improving the design of antigens for vaccines. For example, PRRSV envelope glycoproteins are candidates for use in subunit vaccines (Durand et al., 1997), and the nonstructural ORF 1 polyprotein could be a possible candidate antigen for the development of serological tests to identify carrier animals (Neitzert et al., 1991). Production of full-length recombinant proteins to explore such potential applications may be hampered by the presence of hydrophobic regions and, for the ORF 1 polyprotein, a very large size and the ability to undergo autoproteolytic cleavage. This could be overcome by expression of protein subunits, guided by prior knowledge of naturally antigenic regions. However, no knowledge currently exists about epitopes in the PRRSV ORF 1 nonstructural polyprotein, and only two epitopes have been mapped in the envelope glycoproteins (Meulenberg et al., 1997; Oleksiewicz et al., 2000), one of which was mapped using monoclonal antibodies (MAbs) and thus is of unknown significance for PRRSV infection in vivo (Meulenberg et al., 1997).

References

1. Albina E., Madec F., Cariolet R., and Torrison J., 1994. Immune response and persistence of the porcine reproductive and respiratory syndrome virus in infected pigs and farm units. Vet. Rec. 134:567-573.
2. Albina E., Carrat C., and Charley B., 1998a. Interferon-alpha response to swine arterivirus (PoAV), the porcine reproductive and respiratory syndrome virus. J. Interferon Cytokine Res. 18:485-490.
3. Albina E., Piriou L., Hutet H., and L'Hospitalier R., 1998b. Immune responses in pigs infected with porcine reproductive and respiratory syndrome virus (PRRSV). Vet. Immunol. Immunopathol. 61:49-66.
4. Banyer J.L., Hamilton N.H., Ramshaw I.A., and Ramsay A.J., 2000. Cytokines in innate and adaptive immunity. Rev. Immunogenet. 2:359-373.
5. Bautista E.M., Meulenberg J.J., Choi C.S., and Molitor T.W., 1996. Structural polypeptides of the American (VR-2332) strain of porcine reproductive and respiratory syndrome virus. Arch. Virol. 141:1357-1365.
6. Bautista E.M., and Molitor T.W., 1997. Cell-mediated immunity to porcine reproductive and respiratory syndrome virus in swine. Viral Immunol. 10:83-94.
7. Bautista E.M., Suarez P., and Molitor T.W., 1999. T cell responses to the structural polypeptides of porcine reproductive and respiratory syndrome virus. Arch. Virol. 144:117-134.
8. Benfield D.A., Nelson E., Collins J.E., Harris L., Goyal S.M., Robison D., Christianson W.T., Morrison R.B., Gorcyca D., and Chladek D., 1992. Characterization of swine

infertility and respiratory syndrome (SIRS) virus (isolate ATCC VR-2332). J. Vet. Diagn. Invest. 4:127-33.

9. Benfield D.A., Collins J.E., Dee S.A., Halbure P.G., Joo H.S., Lager K.M., Mengeling W.L., Murtaugh M.P., Rossow K.D., Stevenson G.W., and Zimmerman J.J., 1999. Porcine reproductive and respiratory syndrome virus. In: Straw B.E., D'Allaire S. Mengeling W.L., Taylor D.S., eds. Diseases of Swine. Iowa State University Press, Ames, Iowa, pp. 201-232
10. Brinton M.A., Gavin E.I., and Fernandez A.V., 1986. Genotypic variation among six isolates of lactate dehydrogenase-elevating virus. J. Gen. Virol. 67:2673-2684.
11. Brown I.H., Done S.H., Spencer Y.I., Cooley W.A., Harris P.A., and Alexander D.J., 1993. Pathogenicity of a swine influenza H1N1 virus antigenically distinguishable from classical and European strains. Vet. Rec. 132:598-602.
12. Buddaert W., van Reeth K., and Pensaert M., 1998. In vivo and in vitro interferon (IFN) studies with the porcine reproductive and respiratory syndrome virus (PRRSV). Adv. Exp. Med. Biol. 440:461-467.
13. Bøtner A., Strandbygaard B., Sorensen K.J., Have P., Madsen K.G., Madsen E.S., and Alexandersen S., 1997. Appearance of acute PRRS-like symptoms in sow herds after vaccination with a modified live PRRS vaccine. Vet Rec. 141:497-499.
14. Cavanagh D., 1997. *Nidovirales*: a new order comprising *Coronaviridae* and *Arteriviridae*. Arch. Virol. 142:629-633.
15. Chang C.C., Yoon K.J., Zimmerman J.J., Harmon K.M., Dixon P.M., Dvorak C.M., and Murtaugh M.P., 2002. Evolution of porcine reproductive and respiratory syndrome virus during sequential passages in pigs. J. Virol. 76:4750-4763.

16. Chiou M.T., Jeng C.R., Chueh L.L., Cheng C.H., and Pang V.F., 2000. Effects of porcine reproductive and respiratory syndrome virus (isolate tw91) on porcine alveolar macrophages in vitro. Vet. Microbiol. 71:9-25.

17. Choi C., Christianson W., and Collins J., 1992. Antibody-dependent enhancement of SIRS virus replication. Am. Assoc. Swine Pract. Newsl. 4:30.

18. Christianson W.T., Collins J.E., Benfield D.A., Harris L., Gorcyca D.E., Chladek D.W., Morrison R.B., and Joo H.S., 1992. Experimental reproduction of swine infertility and respiratory syndrome in pregnant sows. Am. J. Vet. Res. 53:485-488.

19. Christianson W.T., Choi C.S., Collins J.E., Molitor T.W., Morrison R.B., and Joo H.S., 1993. Pathogenesis of porcine reproductive and respiratory syndrome virus infection in mid-gestation sows and fetuses. Can. J. Vet. Res. 57:262-268.

20. Christman J.W., Lancaster L.H., and Blackwell T.S., 1998. Nuclear factor kappa B: a pivotal role in the systemic inflammatory response syndrome and new target for therapy. Intensive Care Med. 24:1131-1138.

21. Christman J.W., Sadikot R.T., and Blackwell T.S., 2000. The role of nuclear factor–κB in pulmonary diseases. Chest 117:1482-1487.

22. Christopher-Hennings J., Nelson E.A., Hines R.J., Nelson J.K., Swenson S.L., Zimmerman J.J., Chase C.L., Yaeger M.J., and Benfield D.A., 1995. Persistence of porcine reproductive and respiratory syndrome virus in serum and semen of adult boars. J. Vet. Diagn. Invest. 7:456-464.

23. Christopher-Hennings J., Nelson E.A., Nelson J.K., and Benfield D.A., 1997. Effects of a modified-live virus vaccine against porcine reproductive and respiratory syndrome in boars. Am. J. Vet. Res. 58:40-45.

24. Chung W.B., Lin M.W., Chang W.F., Hsu M., and Yang P.C., 1997. Persistence of porcine reproductive and respiratory syndrome virus in intensive farrow-to-finish pig herds. Can. J. Vet. Res. 61:292-298.

25. Collins J.E., Benfield D.A., Christianson W.T., Harris L., Hennings J.C., Shaw D.P., Goyal S.M., McCullough S., Morrison R.B., and Joo H.S., 1992. Isolation of swine infertility and respiratory syndrome virus (isolate ATCC VR-2332) in North America and experimental reproduction of the disease in gnotobiotic pigs. J. Vet. Diagn. Invest. 4:117-126.

26. Conzelmann K.K., Visser N., Van Woensel P., and Thiel H.J., 1993. Molecular characterization of porcine reproductive and respiratory syndrome virus, a member of the arterivirus group. Virology 193:329-339.

27. Cousens L.P., Peterson R., Hsu S., Dorner A., Altman J.D., Ahmed R., and Biron C.A., 1999. Two roads diverged: interferon α/β-and interleukin 12-mediated pathways in promoting T cell interferon g responses during viral infection. J. Exp. Med. 189:1315-1328.

28. Dea S., Gagnon C.A., Mardassi H., Pirzadeh B., and Rogan D., 2000. Current knowledge on the structural proteins of porcine reproductive and respiratory syndrome (PRRS) virus: comparison of the North American and European isolates. Arch. Virol. 145:659–688.

29. Dee S.A., and Joo H.S., 1994. Recurrent reproductive failure associated with porcine reproductive and respiratory syndrome in a swine herd. J. Am. Vet. Med. Assoc. 205:1017-1018.

30. Dee S.A., Collins J., Halbure P., Keffaber K., Lautner B., McCaw M., Polson D., Rodibaugh M., Sanford E., and Yesek P., 1996. Control of porcine reproductive and respiratory syndrome (PRRS) virus. Swine Health and Production 4:95-98.

31. Dee S.A., Joo H.S., Park B.K., Molitor T.W., and Bruna G., 1998. Attempted elimination of porcine reproductive and respiratory syndrome virus from a seed stock farm by vaccination of the breeding herd and nursery depopulation. Vet. Rec.142:569-572.

32. Durand J.P., Climent I., Sarraseca J., Urinza A., Cortes E., Vela C., and Casal I., 1997. Baculovirus expression of proteins of porcine reproductive and respiratory syndrome virus strain olot/91: involvement of ORF3 and ORF5 proteins in protection. Virus Genes 14:19-29

33. Eichhorn G., and Frost J.W., 1997. Study on the suitability of sow colostrum for the serological diagnosis of porcine reproductive and respiratory syndrome (PRRS). Zentralbl. Veterinarmed. B 44:65-72.

34. Epperson B., and Holler L., 1997. An abortion storm and sow mortality syndrome. Annu. Meet. Am. Assoc. Swine Pract. 28:479-484.

35. Feitsma H., Grooten H.J., Schie F.W.V., and Colenbrander B., 1992. The effect of porcine epidemic abortion and respiratory syndrome (PEARS) on sperm production. In: Proc. 12th International Congress on Animal Reproduction, pp. 1710-1712.

36. Galina L., Pijoan C., Sitjar M., Christianson W.T., Rossow K., and Collins J.E., 1994. Interaction between *Streptococcus suis* serotype 2 and porcine reproductive and respiratory syndrome virus in specific pathogen-free piglets. Vet. Rec. 134:60-64.

37. Gonin P., Pirzadeh B., Gagnon C.A., and Dea S., 1999. Seroneutralization of porcine reproductive and respiratory syndrome virus correlates with antibody response to the GP5 major envelope glycoprotein. J. Vet. Diagn. Invest. 11:20-26.

38. Gorcyca D., Schlesinger K., Chladek D.W., Behan W., Polson D.D., Roof M., and Doitchenoff D., 1995. RespPRRS; a new tool for the prevention and control of PRRS in pigs. In: Proc. 26th Annu. Meet. Am. Assoc. Swine Pract. pp. 1-22.

39. Gorcyca D.E., Schlesinger K.J., Geeding P.W., Chaldek D.W., and Short J.A., 1996. Protection against the reproductive disease caused by porcine productive and respiratory syndrome (PRRS) virus by vaccinating with modified live virus PRRS vaccine prior to breeding. In: Proc. 14th IPVS Congr., Bologna, Italy, p. 66.

40. Gradil C., Dubuc C., and Eaglesome M.D., 1996. Porcine reproductive and respiratory syndrome virus: seminal transmission. Vet. Rec. 138:521-522.

41. Halbur P.G., Paul P.S., Frey M.L., Landgraf J., Eernisse K., Meng X.J., Andrews J.J., Lum M.A., Rathje J.A., 1996. Comparison of the antigen distribution of two US porcine reproductive and respiratory syndrome virus isolates with that of the Lelystad virus. Vet. Pathol. 33:159-170.

42. Halbur P.G., and Bush E., 1997. Update on abortion storms and sow mortality. Swine Health and Production 5:73.

43. Halbur P., Rothschild M., and Thacker B., 1997. Differences in susceptibility of Duroc, Hampshire, and Meishan pigs to infection with a high-virulence strain (VR2385) of porcine reproductive and respiratory syndrome virus (PRRSV). J. Anim. Breed. Genet. 115:181-189.

44. Hill H.T., 1990. Overview and history of mystery swine disease (swine infertility/ respiratory syndrome). In: Proc. Mystery Swine Disease Committee Meeting, Livestock Conservation Institute, Madison, pp. 29-31

45. Hopper S.A., White M.E., and Twiddy N., 1992. An outbreak of blue-eared pig disease (porcine reproductive and respiratory syndrome) in four pig herds in Great Britain. Vet Rec. 131:140-144.

46. Hesse R.A., Counter L.P., Lau M.L., Dimmick S.K., and Ellsworth S.R., 1996a. Efficacy of Prime Pac PRRS in controlling PRRS reproductive disease: Homologous challenge. In: Proc. 27th Annu. Meet. Am. Assoc. Swine Pract. pp. 103-105.

47. Hesse R.A., Counter L.P., Lau M.L., Wunder K.K., and Wasmoen T.L., 1996b. Efficacy of Prime Pac PRRS in controlling PRRS reproductive disease: Heterologous challenge. In: Proc. 27th Annu. Meet. Am. Assoc. Swine Pract. pp. 107-110.

48. Houben S., van Reeth K., and Pensaert M.B., 1995. Pattern of infection with the porcine reproductive and respiratory syndrome virus on swine farms in Belgium. Zentralbl. Veterinarmed. B 42:209-215.

49. Hurd H.S., Bush E., Connor J'Collins J., Epperson B., Gomez T., Kolb J., Lautner B., McCaw M., McKean J., Schmidt B., Schoenbaum M., Terrill M., Wills R., White L., and Zimmerman J., 1998. The acute PRRS investigative study- an update. Am. Assoc. Swine Pract. Newsl. 8:419-421.

50. Joo H.S., Park B.K., Dee S.A., and Pijoan C., 1997. Indirect fluorescent IgM antibody response of pigs infected with porcine reproductive and respiratory syndrome virus. Vet. Microbiol. 55:303-307.

51. Joo H.S., Park B.K., Lee W.H., Shin J.H., Carlson, and Debuse N.K. 1998. Atypical porcine reproductive and respiratory syndrome (PRRS): Clinical investigation and isolation of PRRS virus and a cytopathic agent. In: Proc. 25th IPVS Congr, Birmingham, England, p.125.

52. Jusa E.R., Inaba Y., Kouno M., Hirose O, Shibata I, Kubota M, and Yasuhara H., 1996. Slow-reacting and complement-requiring neutralizing antibody in swine infected with porcine reproductive and respiratory syndrome (PRRS) virus. J. Vet. Med. Sci. 58:749-753.

53. Kadowaki N., Antonenko S., Lau J.Y., and Liu Y.J., 2000. Natural interferon α/β-producing cells link innate and adaptive immunity. J. Exp. Med. 192:219-226.

54. Kapur V., Elam M.R., Pawlovich T.M., and Murtaugh M.P., 1996. Genetic variation in porcine reproductive and respiratory syndrome virus isolates in the Midwestern United States. J. Gen. Virol. 77:1271-1276.

55. Keffaber K.K., 1989. Reproductive failure of unknown etiology. Am. Assoc. Swine Pract. Newsl. 2:1-10.

56. Kuo L.L., Chen Z., Rowland R.R.R., Faaberg K.S., and Plagemann, P.G.W., 1992. Lactate dehydrogenase-elevating virus (LDV): subgenomic mRNAs, mRNA leader and comparison of 3'-terminal sequences of two LDV isolates. Virus Res. 23:55-72.

57. Kurane I., Mady B.J., and Ennis F., 1991. Antibody-dependent enhancement of dengue virus infection. Rev. Med. Virol. 1:211-221.

58. Kwang J., Kim H.S., and Joo H.S., 1994. Cloning, expression, and sequence analysis of the ORF4 gene of the porcine reproductive and respiratory syndrome virus MN-1b. J. Vet. Diagn. Invest. 6:293-296.

59. Kwang J., Zuckermann F., Ross G., Yang S., Osorio F., Liu W., and Low S., 1999. Antibody and cellular immune responses of swine following immunisation with plasmid DNA encoding the PRRS virus ORF's 4, 5, 6 and 7. Res. Vet. Sci. 67:199-201.

60. Labarque G.G., Nauwynck H.J., Van Reeth K., and Pensaert M.B., 2000. Effect of cellular changes and onset of humoral immunity on the replication of porcine reproductive and respiratory syndrome virus in the lungs of pigs. J. Gen. Virol. 81:1327-1334.

61. Lager K.M., Mengeling W.L., and Brockmeier S.L., 1997. Duration of homologous porcine reproductive and respiratory syndrome virus immunity in pregnant swine. Vet. Microbiol. 58:127-133.

62. Lai M.M., 1995. Transcription, replication, recombination, and engineering of coronavirus genes. Adv. Exp. Med. Biol. 380:463-471

63. Le Bon A., Schiavoni G., D'Agostino G., Gresser I., Belardelli F., and Tough D.F., 2001. Type I interferons potently enhance humoral immunity and can promote isotype switching by stimulating dendritic cells in vivo. Immunity 14:461-470.

64. Le Gall A., Albina E., Magar R., and Gauthier J.P., 1997. Antigenic variability of porcine reproductive and respiratory syndrome (PRRS) virus isolates. Influence of virus passage in pig. Vet. Res. 28:247-257.

65. Loemba H.D., Mounir S., Mardassi H., Archambault D., and Dea S., 1996. Kinetics of humoral immune response to the major structural proteins of the porcine reproductive and respiratory syndrome virus. Arch. Virol. 141:751-761.

66. Lopez Fuertes L., Domenech N., Alvarez B., Ezquerra A., Dominguez J., Castro J.M., and Alonso F., 1999. Analysis of cellular immune response in pigs recovered from porcine respiratory and reproductive syndrome infection. Virus Res. 64:33-42.

67. Lopez-Fuertes L., Campos E., Domenech N., Ezquerra A., Castro J.M., Dominguez J., and Alonso F., 2000. Porcine reproductive and respiratory syndrome (PRRS) virus down-modulates TNF-alpha production in infected macrophages. Virus Res. 69:41-46.

68. Loula T., 1991. Mystery pig disease. Agri-Practice 12:23-24.

69. Madsen K.G., Hansen C.M., Madsen E.S., Strandbygaard B., Botner A., and Sorensen K.J., 1998. Sequence analysis of porcine reproductive and respiratory syndrome virus of the American type collected from Danish swine herds. Arch. Virol. 143:1683-1700.

70. Mardassi H., Massie B., and Dea S., 1996. Intracellular synthesis, processing, and transport of proteins encoded by ORFs 5 to 7 of porcine reproductive and respiratory syndrome virus. Virology 221:98-112.

71. Mavromatis I., Kritas S.K., Alexopoulos C., Tsinas A., and Kyriakis S.C., 1999. Field evaluation of a live vaccine against porcine re-productive and respiratory syndrome in fattening pigs. Zentralbl. Veterinarmed. B 46:603-612.

72. Meier W., Wheeler J., and Husmann R.J., 2000. Characteristics of the immune response of pigs to PRRS virus. Vet. Res. 31:41.

73. Meng X.J., Paul P.S., and Halbur P.G., 1994. Molecular cloning and nucleotide sequencing of the 3'-terminal genomic RNA of the porcine reproductive and respiratory syndrome virus. J. Gen. Virol. 75:1795-1801.

74. Mengeling W.L., Vorwald A.C., Lager K.M., and Brockmeier S.L., 1996. Diagnosis of porcine reproductive and respiratory syndrome using infected alveolar macrophages collected from live pigs. Vet. Microbiol. 49:105-115.

75. Mengeling W.L., Lager K.M., and Vorwald A.C., 1997. An update of research at the National Animal Disease Center on current strains of porcine reproductive and respiratory syndrome (PPRS) VIRUS. In: Proc. 24th anniversary Allen D`Leman Swine Conf. U. Minnesota, pp. 138-145

76. Mengeling W.L., Lager K.M., and Vorwald A.C., 1999. Safety and efficacy of vaccination of pregnant gilts against porcine reproductive and respiratory syndrome. Am. J. Vet. Res. 60:796-801.

77. Meredith M.J., 1993. Porcine reproductive and respiratory syndrome. Cambridge University Press, Cambridge.

78. Meulenberg J.J.M., Hulst M.M., De Meijer E.J., Moonen P.L.J.M., Den Besten A., De Kluyver E.P., Wensvoort G., and Moormann R.J.M., 1993. Lelystad virus, the causative agent of porcine epidemic abortion and respiratory syndrome (PEARS), is related to LDV and EAV. Virology 192:62-72.

79. Meulenberg J.J., Petersen-den Besten A., De Kluyver E.P., Moormann R.J., Schaaper W.M., and Wensvoort G., 1995. Characterization of proteins encoded by ORFs 2 to 7 of Lelystad virus. Virology 206:155-163.

80. Meulenberg J.J., and Petersen-den Besten A., 1996. Identification and characterization of a sixth structural protein of Lelystad virus: the glycoprotein GP2 encoded by ORF2 is incorporated in virus particles. Virology 225:44-51.

81. Meulenberg J.J., van Nieuwstadt A.P., van Essen-Zandbergen A., and Langeveld J.P.M., 1997. Posttranslational processing and identification of a neutralization domain of the GP4 protein encoded by ORF4 of Lelystad virus. J. Virol. 71:6061-6067.

82. Molitor T.W., Bautista E.M., and Choi C.S., 1997. Immunity to PRRSV: double-edged sword. Vet. Microbiol. 55:265-276.

83. Morrison R.B., Collins J.E., Harris L., Christianson W.T., Benfield D.A., Chladek D.W., Gorcyca D.E., and Joo H.S., 1992. Serologic evidence incriminating a recently isolated virus (ATCC VR-2332) as the cause of swine infertility and respiratory syndrome (SIRS). J. Vet. Diagn. Invest. 4:186-188.

84. Mounir S., Mardassi H., and Dea S., 1995. Identification and characterization of the porcine reproductive and respiratory virus ORFs 7, 5 and 4 products. Adv. Exp. Med. Biol. 380:317-320.

85. Murtaugh M.P., Elam M.R., and Kakach L.T., 1995. Comparison of the structural protein coding sequences of the VR-2332 and Lelystad virus strains of the PRRS virus. Arc Virol. 140:1451-1460.

86. Murtaugh M.P., Xiao Z., and Zuckermann F., 2002. Immunological Responses of Swine to Porcine Reproductive and Respiratory Syndrome Virus Infection. Viral Immun. 4:533-547.

87. Neitzert E., Beck E., de Mello P.A., Gomes I., and Bergmann I.E., 1991. Expression of the aphthovirus RNA polymerase gene in Escherichia coli and its use together with other bioengineered nonstructural antigens in detection of late persistent infections. Virology 184:799-804.

88. Nelson E.A., Christopher-Hennings J., and Benfield D., 1994. Serum immune response to the proteins of porcine reproductive and respiratory syndrome (PRRS) virus. J. Vet. Diagn. Invest. 6:410-415.

89. Nelson E.A., Christopher-Hennings J., and Benfield D.A., 1995. Structural proteins of porcine reproductive and respiratory syndrome virus (PRRSV). Adv. Exp. Med. Biol. 380:321-323.

90. Nielsen J., and Bøtner A., 1997. Hematological and immunological parameters of 4 1 /2-month old pigs infected with PRRS virus. Vet. Microbiol. 55:289-294.

91. Nielsen T.L., Nielsen J., Have P., Baekbo P., Hoff J.R., and Botner A., 1997. Examination of virus shedding in semen from vaccinated and from previously infected boars after experimental challenge with porcine reproductive and respiratory syndrome virus. Vet. Microbiol. 54:101-112.

92. Oleksiewicz M.B., Botner A., Toft P., Grubbe T., Nielsen J., Kamstrup S., and Storgaard T., 2000. Emergence of porcine reproductive and respiratory syndrome virus deletion mutants: correlation with the porcine antibody response to a hypervariable site in the ORF 3 structural glycoprotein. Virology. 267:135-140.

93. Oleksiewicz M.B., Bøtner A., and Normann P., 2001a. Semen from boars infected with porcine reproductive and respiratory syndrome virus (PRRSV) contains antibodies against structural as well as nonstructural viral proteins. Vet. Microbiol. 81:109-125.

94. Oleksiewicz M.B., Bøtner A., Toft P., Normann P., and Storgaard T., 2001b. Epitope mapping porcine reproductive and respiratory syndrome virus by phage display: the nsp2 fragment of the replicase polyprotein contains a cluster of B cell epitopes. J. Virol. 75:3277-3290.

95. Oleksiewicz M.B., Bøtner A., and Normann P., 2002. Porcine B-cells recognize epitopes that are conserved between the structural proteins of American-and European-type porcine reproductive and respiratory syndrome virus. J. Gen. Virol. 83:1407-1418.

96. Osorio F., Galeota J., Nelson E., Brodersen B., Doster A., Wills R., Zuckermann F., and Laegreid W.W., 2002. Passive transfer of virus-specific antibodies confers protection against reproductive failure induced by a virulent strain of porcine reproductive and respiratory syndrome virus and establishes sterilizing immunity. Virology 302:9-11.

97. Ostrowski M., Galeota J.A., Jar A.M., Platt K.B., Osorio F.A., and Lopez O.J., 2002. Identification of neutralizing and nonneutralizing epitopes in the porcine reproductive and respiratory syndrome virus GP5 ectodomain. J. Virol. 76:4241-4250.

98. Park B.K., Joo H.S., Dee S.A., and Pijoan, C., 1995. Evaluation of an indirect fluorescent IgM antibody test for the detection of pigs with recent infection of porcine reproductive and respiratory syndrome virus. J. Vet. Diagn. Invest.7:544-546.

99. Pastoret P., Griebel P., and Bazin H., 1998. Handbook of Vertebrate Immunology. Academic Press, San Diego.

100. Pfeffer L.M., Dinarello C.A., Herberman R.B., Williams B.R., Borden E.C., Bordens R., Walter M.R., Nagabhushan T.L., Trotta P.P., and Pestka S., 1998. Biological properties of recombinant alpha-interferons: 40th anniversary of the discovery of interferons. Cancer Res. 58:2489-2499.

101. Pirzadeh B., and Dea S., 1997. Monoclonal antibodies to the ORF5 product of porcine reproductive and respiratory syndrome virus define linear neutralizing determinants. J. Gen. Virol. 78:1867-1873.

102. Pirzadeh B., and Dea S., 1998. Immune response in pigs vaccinated with plasmid DNA encoding ORF5 of porcine reproductive and respiratory syndrome virus. J. Gen. Virol. 79:989-999.

103. Plagemann P.G.W., and Moennig B., 1992. Lactate dehydrogenase-elevating virus, equine arteritis virus and simian hemorrhagic fever virus: a new group of positive stranded RNA viruses. Adv. Virus Res. 41:99-192

104. Plana Duran J., Climent I., Sarraseca J., Urniza A., Cortes E., Vela C., and Casal J.I., 1997. Baculovirus expression of proteins of porcine reproductive and respiratory syndrome virus strain Olot/91. Involvement of ORF3 and ORF5 proteins in protection. Virus Genes 14:19-29.

105. Pol J.M., van Dijk J.E., Wensvoort G., and Terpstra C., 1991. Pathological, ultrastructural, and immunohistochemical changes caused by Lelystad virus in experimentally induced infections of mystery swine disease (synonym: porcine epidemic abortion and respiratory syndrome (PEARS)). Vet. Q. 13:137-143.

106. Ramsay A.J., Ruby J., and Ramshaw I.A., 1993. A case for cytokines as effector molecules in the resolution of virus infection. Immunol. Today 14:155-157.

107. Regnier C.H., Song H.Y., Gao X., Goeddel D.V., Cao Z., and Rothe M., 1997. Identification and characterization of an IkB kinase. Cell 90:373-383.

108. Rodriguez M.J., Sarraseca J., Fominaya J., Cortes E., Sanz A., and Casal J.I., 2001. Identification of an immunodominant epitope in the C terminus of glycoprotein 5 of porcine reproductive and respiratory syndrome virus. J. Gen. Virol. 82:995-999.

109. Rossow K.D., Bautista E.M., Goyal S.M., Molitor T.W., Murtaugh M.P., Morrison R.B., Benfield D.A., and Collins J.E., 1994. Experimental porcine reproductive and

respiratory syndrome virus infection in one-, four-, and 10-week-old pigs. J. Vet. Diagn. Invest. 6:3-12.

110. Rossow K., 1997. Investigation of an abortion in a RespPPRS vaccinated swine herd from southeast Iowa. In: Supplement to Proc. 27th Anniversary of Allen D`Leman Swine Confer. U. Minnesota, pp.1-2.

111. Rossow K.D., 1998. Porcine reproductive and respiratory syndrome. Vet. Pathol. 35:1-20.

112. Rowland R.R., Steffen M., Ackerman T., and Benfield D.A., 1999. The evolution of porcine reproductive and respiratory syndrome virus: quasispecies and emergence of a virus subpopulation during infection of pigs with VR-2332. Virology 259:262-266.

113. Rowland R.R., Robinson B., Stefanick J., Kim T.S., Guanghua L., Lawson S.R., and Benfield D.A., 2001. Inhibition of porcine reproductive and respiratory syndrome virus by interferon-g and recovery of virus replication with 2-aminopurine. Arch. Virol. 146:539-555.

114. Sagripanti J.L., 1985. Polyadenylic acid sequences in the genomic RNA of the togavirus of simian hemorrhagic fever. Virology 145:350-355.

115. Sagripanti J.L., Zandomeni R.O., and Weinmann R., 1986. The cap structure of simian hemorrhagic fever virion RNA. Virology 151:146-150.

116. Samsom J.N., de Bruin T.G., Voermans J.J., Meulenberg J.J., Pol J.M., and Bianchi A.T., 2000. Changes of leukocyte phenotype and function in the broncho-alveolar lavage fluid of pigs infected with porcine reproductive and respiratory syndrome virus: a role for CD8 1 cells. J. Gen. Virol. 81:497-505.

117. Schmid R.M., and Adler G., 2000. NF-κB/rel/IκB: implications in gastrointestinal diseases. Gastroenterology 118:1208-1228.

118. Shibata I., Mori, M., and Uruno K., 1998. Experimental infection of maternally immune pigs with porcine re-productive and respiratory syndrome (PRRS) virus. J. Vet. Med. Sci. 60:1285-1291.

119. Shimizu M., Yamada S., Kawashima K., Ohashi S., Shimizu S., and Ogawa T., 1996. Changes of lymphocyte subpopulations in pigs infected with porcine reproductive and respiratory syndrome (PRRS) virus. Vet. Immunol. Immunopathol. 50:19-27.

120. Smith S.L., Wang X.C., and Godeny E.K., 1997. Sequence of the 3' end of the simian hemorrhagic fever virus genome. Gene 191:205-210.

121. Stevenson G.W., Van Alstine W.G., Kanitz C.L., and Keffaber K.K., 1993. Endemic porcine reproductive and respiratory syndrome virus infection of nursery pigs in two swine herds without current reproductive failure. J. Vet. Diagn. Invest. 5:432-434.

122. Storgaard T., Oleksiewicz M., and Bøtner A., 1999. Examination of the selective pressures on a live PRRS vaccine virus. Arch. Virol. 144:2389-2401.

123. Suarez P., 2000. Ultrastructural pathogenesis of the PRRS virus. Vet. Res. 31:47-55.

124. Takikawa N., Kobayashi S., Ide S., Yamane Y., Tanaka Y., Higashihara M., and Yamagishi H., 1997. Early serodiagnosis of porcine reproductive and respiratory syndrome virus infection of pigs by detection of slow-reacting and complement-requiring neutralizing antibody. J. Vet. Med. Sci. 59:31-34.

125. Terpstra C., Wensvoort G., and Pol J.M., 1991. Experimental reproduction of porcine epidemic abortion and respiratory syndrome (mystery swine disease) by infection with Lelystad virus: Koch's postulates fulfilled. Vet. Q. 13:131-136.

126. Thacker E.L., Halbur P.G., Ross R.F., Thanawongnuwech R., and Thacker B.J., 1999. Mycoplasma hyopneumoniae potentiation of porcine reproductive and respiratory syndrome virus-induced pneumonia. J. Clin. Microbiol. 37:620-627.

127. Thanawongnuwech, R., T.F. Young, B.J. Thacker, et al. 2001. Differential production of proinflammatory cytokines: in vitro PRRSV and Mycoplasma hyopneumoniae co-infection model. Vet. Immunol. Immunopathol. 79:115-127.

128. Tizard I.F., 1995. Interferons. In: M.J. Myers, and M.P. Murtaugh (eds.), Cytokines in Animal Health and Disease. Marcel-Dekker, New York, pp. 1-58.

129. Tough D.F., Sun S., Zhang X., and Sprent J., 1999. Stimulation of naive and memory T cells by cytokines. Immunol. Rev. 170:39-47.

130. van Berlo M.F., Horzinek M.C., van der Zeijst B.A., 1982. Equine arteritis virus-infected cells contain six polyadenylated virus-specific RNAs. Virology 118:345-352.

131. van Nieuwstadt A.P., Meulenberg J.J., van Essen-Zanbergen A., Petersen-den Besten A., Bende R.J., Moormann R.J., and Wensvoort G., 1996. Proteins encoded by open reading frames 3 and 4 of the genome of Lelystad virus (Arteriviridae) are structural proteins of the virion. J. Virol. 70:4767-4772.

132. van Reeth K., Labarque G., Nauwynck H., and Pensaert M., 1999. Differential production of proinflammatory cytokines in the pig lung during different respiratory virus infections: correlations with pathogenicity. Res. Vet. Sci. 67:47-52.

133. van Reeth K., and Nauwynck H., 2000. Proinflammatory cytokines and viral respiratory disease in pigs. Vet. Res. 31:187-213.

134. van Woensel P.A., Liefkens K., and Demaret S., 1998. Effect on viremia of an American and a European serotype PPRSV vaccine after challenge with European wild-type strains of the virus. Vet. Rec. 142:510-512.

135. Vicek J., and Sen G., 1996. Interferons and other cytokines. In B. Fields, D. Knipe, and P. Howley (eds.), Fields' Virology, 3rd ed. Vol. 13, Lippincott–Raven Publishers, Philadelphia, pp. 375-400

136. Weiland E., Wieczorek-Krohmer M., Kohl D., Conzelmann K.K., and Weiland F., 1999. Monoclonal antibodies to the GP5 of porcine reproductive and respiratory syndrome virus are more effective in virus neutralization than monoclonal antibodies to the GP4. Vet. Microbiol. 66:171-186.

137. Wensvoort G., Terpstra C., Pol J.M., ter Laak E.A., Bloemraad M., de Kluyver E.P., Kragten C., van Buiten L., den Besten A., Wagenaar F., 1991. Mystery swine disease in The Netherlands: the isolation of Lelystad virus. Vet. Q. 13:121-130.

138. Wensvoort G., 1993. Lelystad virus and the porcine epidemic abortion and respiratory syndrome. Vet. Res. 24:117-124.

139. White M., 1991. Letter to the editor. Vet. Rec. 128:574.

140. Wills R.W., Zimmerman J.J., Yoon K.J., Swenson S.L., McGinley M.J., Hill H.T., Platt K.B., Christopher-Hennings J., and Nelson E.A., 1997. Porcine reproductive and respiratory syndrome virus: a persistent infection. Vet. Microbiol. 55:231-240.

141. Yang L., Frey M.L., Yoon K.-J., Zimmerman J.J., and Platt K.B., 2000. Categorization of North American porcine reproductive and respiratory syndrome viruses: epitopic profiles of the N, M, GP5 and GP3 proteins and susceptibility to neutralization. Arch. Virol. 145:1599-1619.

142. Yoon I.J., Joo H.S., Christianson W.T., Kim H.S., Collins J.E., Carlson J.H., and Dee S.A., 1992. Isolation of a cytopathic virus from weak pigs on farms with a history of swine infertility and respiratory syndrome. J. Vet. Diagn. Invest. 4:139-143.

143. Yoon I.J., Joo H.S., Goyal S.M., and Molitor T., 1994. A modified serum neutralization test for the detection of antibody to porcine reproductive and respiratory syndrome virus in swine sera. J. Vet. Diagn. Invest. 6:289-292.

144. Yoon K.-J., Wu L.L., Zimmerman J.J., Swenson S.L., McKinley M.J., Landgraf J., Frey M.L., Hill H.T., and Platt K.B., 1995. Characterization of the humoral immune response to porcine reproductive and respiratory syndrome (PRRS) virus infection. J. Vet. Diagn. Invest. 7:305-312.

145. Yoon K.J., Wu L.L., Zimmerman J.J., Hill H. T., and Platt K. B., 1996. Antibody-dependent enhancement (ADE) of porcine reproductive and respiratory syndrome virus (PRRSV) infection in pigs. Viral Immunol. 9:51-63.

146. Yoon K.J., Wu L.L., Zimmerman J.J., and Platt K.B., 1997. Field isolates of porcine reproductive and respiratory syndrome virus (PRRSV) vary in their susceptibility to antibody dependent enhancement (ADE) of infection. Vet. Microbiol. 55:277-287.

147. Zhang X., Shin J., Molitor T.W., Schook L.B., and Rutherford M.S., 1999. Molecular responses of macrophages to porcine reproductive and respiratory syndrome virus infection. Virology 262:152-162.

148. Zimmerman J.J., Yoon K.J., Wills R.W., and Swenson S.L., 1997. General overview of PRRSV: a perspective from the United States. Vet. Microbiol. 55:187-196.

149. Zimmerman J.J., 1999. What we know about persistent infection with respect to the epidemiology of PRRS virus. In: Proc. 30th Annu. Meet. Am. Assoc. Swine Pract. pp. 311-312.

150. Zinkernagel R.M., Bachmann M.F., Kundig T.M., Oehen S., Pirchet H., and Hengartner H., 1996. On immunological memory. Annu. Rev. Immunol. 14:333-367.

151. Zuckermann F.A., and Husmann R.J., 1996. Functional and phenotypic analysis of porcine peripheral blood CD4/CD8 double-positive T cells. Immunology 87:500-512.

152. Zuckermann F.A., Husmann R.J., Schwartz R., Brandt J., Mateu de Antonio E., and Martin S., 1998. Interleukin-12 enhances the virus-specific interferon gamma response of pigs to an inactivated pseudorabies virus vaccine. Vet. Immunol. Immunopathol. 63:57-67.

153. Zuckermann F.A., Martin S., Husmann R.J., and Brandt J., 1999. Use of interleukin 12 to enhance the cellular immune response of swine to an inactivated herpesvirus vaccine. Adv. Vet. Med. 41:447-461.

Part II: Idiotypes and Anti-idiotypic Antibodies

History of Anti-idiotypic antibodies

An antibody is usually defined in terms of the antigen it recognizes. An antibody's specificity for a particular antigen is determined by its antigen-binding site, the distinct region of the antibody molecule that makes contact with an antigen. This site is found within the variable (V) regions of immunoglobulin heavy (VH) and light (VL) chains.

However, an antibody may also be defined by its idiotopes, or surface markers, associated with the unique VH and VL regions of a monospecific population of antibody molecules. Idiotopes may be either in the antigen-binding site or on the framework regions of the variable domain. There are usually several idiotopes in each V domain, some unique to that antibody (private idiotopes), and others shared with some other antibodies (public idiotopes). Public idiotopes are thought to be derived from germ-line gene products (Dreesman and Kennedy, 1985). The sum of the idiotopes on an antibody's V domain determines its idiotype (Id). Idiotypes are useful markers because they enable researchers to follow the appearance and persistence of particular antibodies in immune responses and inherited immunoglobulin genes (Green and Nisonoff, 1984). Ids are also unique determinants that can stimulate production of antiidiotype (anti-Id) antibodies.

The possibility that an antibody might be produced against the combining region of another antibody was suggested by Ehrlich and Morgenroth (Oudin and Michel, 1963). They proposed that autoimmunity to red blood cells was prevented by the formation of an auto anti-lysin that combined with and inactivated lytic antibodies to red cells. Their hypothesis was actually closely related to the "internal image" concept later elaborated by Niels Jerne

(discussed below); because they postulated that the anti-antibody had combining groups or "side chains" equivalent to side chains on red cells. The lysin was therefore able to induce the secretion of anti-antibodies through interaction with the same side chains that are present on red cells. Virtually nothing was known, of course, about the chemical nature of an antibody. The first studies to characterize idiotypic determinants on antibody molecules were performed by Oudin and Michel, and by Kunkel et al. in 1963 (Kunkel et al., 1963; Oudin and Michel, 1963). These studies examined antisera generated against homogeneous immunoglobulins (from multiple myelomas and Bence Jones proteins) and revealed the antigenic individuality of immunoglobulin molecules. In 1974, Neils Jerne proposed a biological significance for idiotypic determinants in his network theory of immune regulation. Jerne theorized that an immune response to a given antigen is controlled by a series of Id-anti-Id reactions that may either enhance or suppress an immune response (Jerne, 1974). The antibody made in response to the original antigen itself becomes an antigen and elicits the synthesis of a second, anti idiotypic antibody. Thus, a given Id (Ab1) is under control of an anti-Id (Ab2), and the anti-Id may be regulated by another set of antibodies referred to as anti-anti-Id (Ab3). According to this original network hypothesis, the Id-anti-Id interactions regulate the immune response of a host to a given antigen. Jerne's network theory successfully explained the appearance of "anti antibodies" previously noted by many laboratories. As a result, new emphasis was placed on self-recognition as an essential component of immune regulation.

Classes of Anti idiotypic Antibodies

A heterogeneous population of anti-Id is obtained when syngeneic inbred animals are immunized with Id. Anti-Ids can be serologically classified into four categories (Ab2α, Ab2ß, Ab2γ and Ab2ε) (Bona and Köhler, 1984; Jerne, 1974; Jerne et al., 1982). The parameters used to define each anti-Id focus primarily on whether it binds within the antigen-binding site or to some other region of the Id (Fig.1). If the relevant antigen or hapten inhibits the binding of anti-Id to Id, the target idiotope is believed to be in the antigen-binding site, and the anti-Id is referred to as Ab2ß. Anti-Ids that are not inhibited by antigen are designated Ab2α and presumably bind to sites in the framework region. Ab2γ recognizes an Id within the antigen-combining site but does not carry the internal image of the original antigen. Ab2γ may partially block Ag-Ab1 binding due to steric hindrances or allosteric effects. Ab2ε is defined as the antibody that recognizes the Id of Ab1 and an epitope on the antigen (Bona and Köhler, 1984).

Internal image antibodies

Ab2ß that can displace an antigen from the antigen-binding site of an antibody may "look" like the antigen and are referred to as internal image anti-Id. It is therefore important to define the criteria for internal imagery. The first criterion is the ability to mimic antigen. An Ab2ß must be capable of inducing an antibody response of the same specificity as the antigen it mimics or to act like a natural ligand for a cellular receptor. This functional criterion also suggests that internal images can induce T cell mediated functions via interactions with T cell receptors. The second criterion is the ability to function as antigen in a variety of animal

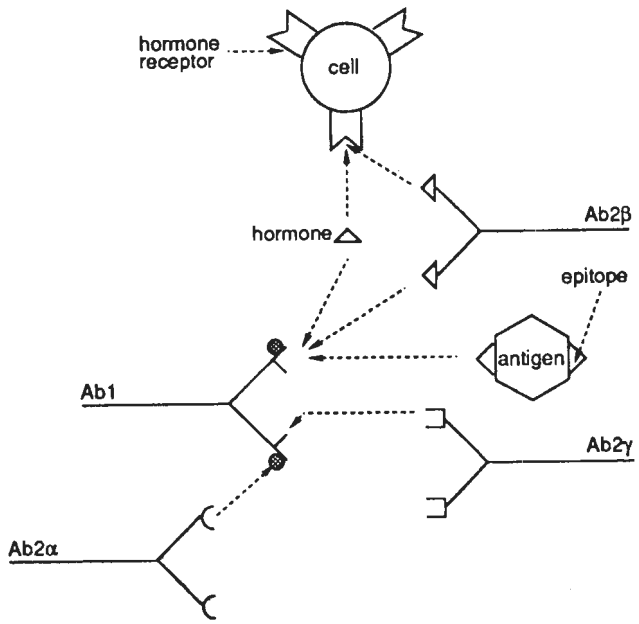

Figure 1. Interactions of different Ab2 with Ab1 (Green and Nisonoff, 1984).

species or inbred strains. This criterion is a means of distinguishing Ab2ß from Ab2α, Ab2 or Ab2ε.

Internal imagery of anti-Ids was first reported when anti-Ids raised against antibodies to insulin reproduced certain physiological reactions of the hormone itself when bound to insulin receptors (Sege and Peterson, 1978). Subsequently, anti-Id mimicry of bacterial, viral, parasitic, and tumor antigens proved successful in generating experimental anti-Id vaccines (Kennedy and Dreesman, 1986; Langone, 1989). All evidence to date indicates that anti-Id mimicry of antigens is due to similarities in three-dimensional structural conformations and, in some cases, to regions of DNA-sequence homology between antigen and anti-Id. Crystallographic analysis of a lysozyme-Ab1 Fab complex and an Ab1 Fab-Ab2 Fab complex showed that the anti-Id antibody and the antigen completely overlapped in their

binding to the Ab1 (Bentley et al., 1990). Moreover, it has been shown that the interacting molecular surfaces of the two Id-anti-Id Fab fragments greatly resembled the structural features of a typical antigen-antibody complex (Ban et al., 1994). Sequence homology between anti-Id and antigen has been clearly demonstrated for reovirus type 3 hemagglutinin as well (Williams et al., 1989). In fact, the V_H and V_L complementarity determining region 1s (CDR1s) of the anti-Id showed sequence homology with regions of the antigen, suggesting that these are the sequences and structures of the external antigen mimicked by the anti-Id.

Whether an anti-Id can accurately mimic an antigen depends greatly on its chemical nature. In the most common antigens such as proteins, peptides, small haptens, and carbohydrates, the groups forming the major epitopes are chemically dissimilar and present different problems for mimicry by anti-Id (Amzel et al., 1994). Complex antigens, including tumor antigens, contain multiple epitopes. The immune recognition which they evoke, therefore, contains many idiotopes which are determined not only by the heterogeneity of the epitopes, but also by the heterogeneity of the V genes selected among Ig and T cell receptor genes in the host. These idiotopes are largely defined by the anti-idiotopes which they induce. Thus private idiotopes elicit Ab2 responses which are unique for a particular Ab1, public idiotopes elicit Ab2 to a specificity shared by many Ab1, and Ab2 of the internal image type are induced against antigen-binding structures on Ab1 which are complementary to the antigen (Augustin et al., 1983; Mosier and Feeney, 1984; Urbain et al., 1982).

Ab2 immunization can lead to Ab3 elicitation not only through specific V gene network interactions but also by virtue of internal-image mimicry (Augustin et al., 1983; Urbain et al., 1982). That is, when the anti-idiotype represents the conformational mirror image of the

antigen, it can substitute for nominal antigen and elicit an Ab1-like response (Nisonoff and Lamoyi, 1981). In an embodiment of the invention employing monoclonal anti-idiotypes, the appropriate Ab2 should be carefully selected. The experimental verification that any particular Ab2 is an internal image type of anti-idiotype hinges on its ability to mimic the conformational characteristics which define recognition of the antigen. Internal image anti-idiotypes compete *in vitro* with antigen for binding to idiotype-positive Ab1, and prime in vivo for Ab3 which mimic Ab1 (Nisonoff and Lamoyi, 1981). In addition to the inhibition of Ab1 binding to antigen, internal image Ab2 can substitute for antigen in terms of immune recognition. For example, Ab2 may stimulate antigen-specific clones *in vitro* in the absence of antigen, or Ab2+ cells may serve as a target for antigen-specific CTL (Ertl et al., 1982).

The ability of internal image Ab2 to compete with antigen for binding to Ab1 (and vice versa) is an integral part of their behavior. However, Ab2 which do not function as internal images may still compete, due to steric hindrance (and perhaps other mechanisms as well), so anti-idiotypic antibodies which do not have internal image specificity may still display internal image-associated properties (Lee et al., 1986; Schick et al., 1987).

If one takes into account both the specificity and idiotypic characteristics of Ab1 molecules, four discrete types of Ab3 molecules may be anticipated: Id- Ag-, Id+ Ag-, Id- Ag+, Id+ Ag+ (Corbet et al., 1988). The latter includes antibodies that resemble Ab1 by reacting with the same antigen: they are designated Ab1' and the Ab2 inducing them is termed the internal image of the antigen. Anti-Ids have been used to raise antibodies against various pathogens and tumor antigens and have often been discussed as possible vaccines. The relationship between Ab1 and Ab1' antibodies has been explored in a number of different antigenic

systems, and with either monoclonal or polyclonal anti-Id reagents (Bhattacharya-Chatterjee et al., 2000). One result obtained consistently is that among Ab3 antibodies are some which do indeed closely resemble the Ab1 in that they possess the same idiotopes, bind to the same antigen and derive from similar variable (V) genes. However, while the resemblance between Ab1' and Ab1 can be very close, many permutations of results have been seen. For example, binding of Ab3 to the antigen may not occur even though the same V-gene segments are selected, e.g. where the selection by anti-Id is directed towards the V segment rather than D and J idiotopes; or the affinity of Ab1' antibodies for antigen may be lower than that of the Ab1, corresponding to differences in the complementarity determining region (CDR), particularly H3 sequences; or the Ab3 may bind a similar antigen, yet diverge considerably from the Ab1 in V genes and CDRs. Thus, depending perhaps on the nature of the Ab2 reagent, an Ab3 population can be more or less heterogeneous and include molecules with a range of fidelities to the Ab1.

According to Goldbaum et al. (1997), there are two major possibilities for the induction of anti-anti-Id (Ab3) that resemble Ab1. One is that a suitably selected anti-Id will mimic the antigen so closely as to produce the equivalent of an antibody response to the original antigen. A second possibility is that the anti-Id is specific for the Id and will thus stimulate lymphocytes displaying anti-anti-Id immunoglobulins that should be identical or essentially identical to the Ab1. By immunochemical criteria it is difficult to discriminate between Ab2β and Ab2γ. To sort out both types of Ab2 a functional demonstration should be observed. Since Ab2β express an idiotype that mimics the antigen, it might have the capacity to elicit antibodies specific for the antigen in the same or different species. The concept of internal

image stems from the possibility of raising Ab3 antibodies that would recognize the original Ag (Jerne et al., 1982).

Application of anti-idiotypic Antibodies

Anti-idiotypic antibodies, and especially those that mimic the antigen or ligand (Ab2β), are being explored in the development of new vaccines, the treatment of cancers (especially T and B cell cancers), hormone or ligand mimicry and as immunodiagnostic reagents.

1. Anti-idiotypic Vaccines

The network hypothesis offers an elegant concept for developing vaccines that are not based on the conventional approach of using nominal antigen. These so-called anti-Id vaccines or internal Ag vaccines take advantage of the fact that the repertoire of external or nominal antigens is mimicked by Id structures on immunoglobulins and possibly on receptors and products of T cells as well. Thus, with this approach, Id-based vaccines do not contain nominal Ag or its fragments. The ability of Ab2α and Ab2β to induce the immune responses (both antibody and T cell responses) to a given pathogen has been shown in many model systems including viruses (Dinca et al., 1993; Francotte and Urbain, 1984; Kennedy and Dressman, 1984; Kennedy and Dressman, 1986; Kennedy et al., 1986; Oosterlaken et al., 1991; Orten et al., 1991; Schick et al., 1987; Zhou et al., 1987; Zhou et al., 1990), bacteria (Kaufmann, 1985; McNamara et al., 1984; Su et al., 1992), and parasites (Kresina and Olds, 1989; Sacks and Sher, 1983; Velge-Roussel et al., 1989).

Vaccinating with anti-idiotypic antibodies (Ab2β) (that carry the internal image of the antigen) offer a number of advantages over immunizing with conventional vaccines. Anti-idiotypic antibodies are not infectious yet can generate an immune response against the pathogen. In addition, anti-idiotypic antibodies induce a more effective T cell response than inactivated viruses do. Also, they are useful when it is difficult or hazardous to obtain large quantities of the antigen. It is thought that they diminish the potential of autoimmune diseases arising as a result of antibodies that are generated to other epitopes on the antigen or other antigens on the viral coat (Dreesman and Kennedy, 1985). They may also prove to be beneficial when the epitopes is a carbohydrate, lipid, or DNA (since these epitopes may be more difficult to mass produce than an anti-idiotypic antibody protein). This may be useful in developing vaccines for parasitic infections (Burdette and Schwartz, 1987; Eichmann and Rajewsky, 1975; Kearney, 1989). Rodent anti-idiotypic vaccines for immunization against a number of pathogens have been developed. For bacteria, there are anti-idiotypic vaccines against *Escherichia coli*, *Streptococcus pneumoniae*, and *Brucella abortus*. Both the idiotypic (Ab1) and the anti-idiotypic (Ab2) antibody were capable of immunizing mice against *E. coli* (Beauclair and Khansari, 1990; McNamara et al., 1984). Rodent anti-idiotypic vaccines have been generated against the following viruses: murine leukemia virus, murine mammary tumor virus (MMTV), reovirus, poliovirus, rabies virus, hepatitis B virus, and the human immunodeficiency virus (HIV) (Burdette and Schwartz, 1987; Kennedy et al., 1986; Thanavala et al., 1986; Thanavala and Roitt, 1986). Immunization with anti-idiotypic antibodies against MMTV induced both cellular and humoral immunity in the mice. The internal image Ab2β antibodies have been shown to induce specific immune responses to hepatitis B surface antigen in different species (Kennedy and Dressman, 1984; Kennedy and

Dressman, 1986; Kennedy et al., 1986; Thanavala et al., 1985). Anti-idiotypic antibodies may prove useful in developing vaccines against the AIDS virus (HIV). Anti-idiotypic antibodies have been generated that can induce anti-HIV responses specific for glycoproteins on HIV (gp41, gp120, and gp160) (Zhou et al., 1987; Zhou et al., 1990). Tackaberry et al. have shown an anti-Id antibody that mimicked a neutralizing epitope on the glycoprotein b complex of human cytomegalovirus and acted as a network antigen for inducing a specific anti-human cytomegalovirus antibody response (Tackaberry et al., 1993). Jackson et al. (1990) have demonstrated that it is possible to expand the use of anti-idiotypic vaccines for diseases in which the primary target is a mucosal tissue, such as the lung or gut, by using oral immunization.

In addition to protein antigens, anti-idiotypic antibodies may prove exceptionally beneficial in developing vaccines against carbohydrate, lipid, or nucleic acid epitopes (or even drugs). In these cases, the anti-idiotypic antibody is a protein that mimics a nonprotein epitope. For example, rodent anti-idiotypic antibodies have been generated to the parasites *Trypanosoma rhodesiense*, *Trypanosoma cruzi*, and *Shistosoma mansoni* (experimental schistosomiasis). As previously mentioned, parasites often have carbohydrate epitopes, limiting the development of vaccines by busing recombinant technology. Anti-idiotypic antibodies may circumvent this problem (Burdette and Schawartz, 1987; Grzych et al., 1985; Sacks et al., 1985). Anti-idiotypic antibodies have also been made against lipid epitopes. The thymus-independent antigen lipid A [from *Eikenella corrodens* lipopolysaccharide (LPS)], was converted into a thymus-dependent immunogen by developing an anti-idiotypic antibody

against lipid A. Mice previously immunized with the anti-idiotypic antibodies survived when they were given a lethal dose of LPS (Kato et al., 1990).

Anti-idiotypic vaccines also have some potential complications. It is unknown how long anti-idiotypic immunity will last. As with other therapeutic or diagnostic antibodies, humans may develop an anti-rodent monoclonal antibody response against the anti-idiotypic antibodies. Humanization of the anti-idiotypic antibodies by recombinant DNA techniques may be necessary to prevent this. Also, care must be taken when choosing the antibody for the vaccination, since different anti-idiotypic antibodies raised against the same idiotope can have different or even opposite physiological effects. For example, anti-idiotypic antibodies (Ab2) were raised against an antibody (Ab1) reactive with a carbohydrate moiety on group A streptococci. Injection of Ab2 of IgG1 class protected the mice against infection by group A streptococci; both humoral and cell-mediated immunity were induced. In contrast, injection of Ab2 of the IgG2 class suppressed the humoral immune response against the bacteria (Dreesman and Kennedy, 1985; Eichmann and Rajewsky, 1975).

2. Idiotypic manipulation of tumor immunity

The administration of anti-Id antibodies as surrogate tumor-associated antigens represents another potential application of the Id vaccine concept. Both T cells and B cells express idiotopes on their antigen-specific receptors (the T cell receptor or surface Ig receptor). In fact, T cell receptors and Ig receptors can even share idiotopes. Theoretically, anti-idiotypic antibodies against unique idiotopes on T or B cell cancers (such as lymphomas, leukemias, or myelomas) can be used to eradicate the cancer. In a specific embodiment of the invention,

internal image antibodies with tumor antigen activity can be used as tumor "vaccines" towards induction of specific tumor immunity. For example, such vaccines can be therapeutically valuable for patients whose primary neoplasms have been removed but who are at risk for development of metastases. One of the major problems of cancer therapy is immune tolerance, which can be easily overcome by an appropriate anti-Id vaccine as compared to a typical multivalent vaccine consisting of whole cells, lysates or antigen-rich supernatant (Bhattacharya-Chatterjee et al., 2000). Several idiotypic manipulations of tumor immunity have been reported and anti-tumor immunity has even been elicited in experimental animals by injecting with anti-idiotypic expressing internal images of putative tumor-specific antigens (Bona, 1989; Victor-Kobrin et al., 1989). Nepom et al. (1984) described induction of tumor immunity where an oncofetal antigen was introduced into a xenogeneic host. Polyclonal anti-idiotypic antibodies were prepared which recognized idiotypic determinants on a mouse antibody to the p97 c epitope of human melanoma antigen p97. The polyclonal antisera could induce, in mice, both CMI and an Ab3 response to p97. Forstrom et al. (1983) used an anti-idiotypic antibody to induce CMI in mice to a syngeneic chemically induced sarcoma. In this study, the anti-idiotypic antibody was an autoantibody produced by hyperimmunization to the tumor, and the tumor antigen was not a defined molecule. Several studies demonstrated idiotypic manipulations of tumor immunity in syngeneic systems, with undefined antigen molecules (Binz et al., 1982). Flood et al. (1980) showed evidence that murine anti-idiotypic T lymphocytes could participate in an autoimmune reaction to fibrosarcoma-specific T lymphocytes, and thus adversely affect an individual's immune response to a tumor. Binz et al. (1982) used anti-idiotypic antibodies to induce *in vitro* proliferation of T lymphocytes specifically cytotoxic to rat sarcoma cells.

Anti-Id responses have been implicated in the induction of anti-tumor immunity to colorectal cancer (Koprowski et al., 1984). Clinical trials in human colorectal patients with a polyclonal anti-Id raised against the MAb 17-1A that recognizes a colon cancer-associated Ag have shown anti-tumor antibody responses (Herlyn et al., 1987). In another study, it was demonstrated that intradermal injection of 2 mg of anti-Id MAb MK2-23, which mimics a high molecular weight human melanoma antigen, elicited anti-tumor antibody responses in melanoma patients (Mittelman et al., 1992). Additional studies have looked at the effect of anti-idiotypic antibodies on tumor growth. Tilkin et al. (1981) showed that immunization of mice with lymph node cells sensitized to an unidentified sarcoma antigen resulted in tumor rejection and growth inhibition. Kennedy et al. (1985) described the suppression of tumor formation in mice challenged with SV40-transformed cells, after injection with polyclonal anti-idiotypic antibodies related to the SV40 antigen. Koprowski et al. (1984) showed the presence of anti-idiotypic antibodies in patients who had a remission of carcinoma after administration of a monoclonal antibody directed against human gastrointestinal cancer.

In an analysis of idiotopes associated with the response to MCA-induced mouse sarcomas or carcinomas (Lee et al., 1986), immunization with monoclonal Ab2 failed to generate Ab3 with Ab1-like specificity, whereas antitumor T helpe cell was easily induced. This observation, coupled with the finding of idiotype-positive T helper cell in the naturally occurring antitumor response, supports a model in which there can be a direct regulatory interaction between Id+ T cells and anti-idiotypic B cells (and Ab2) (Bismuth, et al., 1984; Nelson and Nepom, 1986; Thomas et al., 1983). In vivo, the Id+ T cells can provide the stimulus for production of anti-idiotype. The lack of Id+ B cell recognition in certain cases

may reflect a defect in the genetic capacity to generate antitumor idiotopes on immunoglobulin molecules; alternatively, the regulatory state in the tumor-bearing host may effectively suppress Id+ Ab1. In addition to idiotypic interactions which result in a T helper cell response leading to tumor rejection, exposure to tumor antigen (or antigen-antibody complexes) can lead to the generation of antigen-specific suppressor T cells (T_S). In several tumor systems, suppressor T cells have been shown to function as inducer cells, triggering and amplifying tumor antigen-specific suppression (Nepom et al., 1983).

In a study on the analysis of Igh-restricted T cell responses to the hapten azobenzenearsonate, the nature of immunoglobulin idiotopes was found to determine the development of T S idiotopes (Hayglass et al., 1986). T cells developing in Igh congenic mice acquired the idiotypic repertoire of the host, and treatment of neonatal animals with antibodies to the immunoglobulin chain abolished the establishment of a normal repertoire of functional T cell idiotypes. Thus, the immunoglobulin compartment could determine the development of complementary T cell recognition elements.

3. Reactions of Anti-Id antibodies with cellular receptors

The anti-idiotypic approach is a powerful strategy that allows the construction of receptor-directed probes without prior isolation of the target receptor. This approach has interested many investigators who study the properties of cell-surface receptors. A major reason is that it is possible, by this method, to generate antireceptor antibodies without using the receptor as the immunogen (Erlanger et al., 1986). Because receptors are often difficult to isolate in sufficient quantity for use as an immunogen this alternative approach has a very practical

application (Nisonoff, 1991). The approach has been successfully used for a wide spectrum of recipient proteins including hormone receptors (Linthicum et al., 1988), ion channels (Kleyman et al., 1991), coagulation factors (Vogel et al., 1990), transport proteins (Pain et al., 1990), and retention proteins (Vaux et al., 1990). In particular, the anti-idiotypic route has proven useful for many receptors of biologically active peptides including insulin (Sege and Peterson, 1978), thyroid-stimulating hormone (Frid et al., 1982), angiotensin II (Couraud, 1987), β-endorphin (Gramsch et al., 1988), substance P (Couraud et al., 1985), morphine (Glasel, 1989), and bradykinin (Hassemann et al., 1991) and pathogens including reovirus (Gaulton et al., 1985; Nepom et al., 1982;), leukemogenic retrovirus (Ardman et al., 1985), and herpes simplex (Huang et al., 1996).

4. Anti-id antibodies as diagnostic reagents

Even though the notion of antigen mimicry by anti-Id has been confirmed by several laboratories for numerous diverse antigens, the utility of anti-Ids as surrogate antigens in diagnostic immunoassays has received little attention. Nevertheless, they are extremely well suited to this application for both competitive immunoassay formats and direct serologic assays for specific antibody. Anti-Ids are promising alternatives to the many antigens that are not appropriate for the large-scale production and/or purification required for components of commercial immunoassays.

To overcome the limitations of conventional antigens, internal image anti-Ids can be used in place of antigen in competitive immunoassays (Jiang et al., 2003; Potocnjak et al., 1982; Zhou and Kisil, 1995). Anti-Ids, like any antibody, can be safely produced and purified in

large quantities, can have multiple sites for the attachment of label, can be labeled without substantially diminishing their stability or ability to compete with antigen, and can be bound to a solid support without appreciable loss of immunoreactivity (Walter et al., 1988). For many of the same reasons, anti-Ids are also well suited for use in noncompetitive serologic assays as direct replacements for native antigen in tests for the presence of specific antibody (Lin and Zhou, 1995; Zhou, 1999). Anti-idiotypic antibodies have been used as serodiagnostic reagents to detect antibodies against viruses such as hepatitis B virus (Irshad et al., 1987; Kennedy and Dressman, 1983; Kim et al., 1989), human cytomegalovirus (Tackaberry et al., 1993), psuedorabies virus (Zhou et al., 1994), bluetongue virus (Zhou and Huang et al., 1995; Zhou and Lin, 1997; Zhou, 1999), influenza virus (Betakova et al., 1998), PRRSV (Jiang et al., 2003, Zhou et al., 2004); bacteria such as *Mycoplasma capricolum* (Benguric et al., 2001), *Vibrio anguillarum* (Yongjuan et al., 2002), parasites such as *Schistosoma mansoni* (Percy and Harn, 1988), *Trypanosoma cruzi* (Reis et al., 1993), *Entamoeba histolytica* (Chavez-Rueda et al., 2002), bovine cysticercosis (Hayunga et al., 1992), hormones such as insulin (Casiglia et al., 1991) and allergens such as ryegrass pollen allergen (Zhou et al., 1991).

It is not unreasonable to ask whether autologous anti-Id that may be present in blood or serum samples would interfere in anti-Id-based immunoassays. Data from early experiments on auto anti-idiotypic antibodies indicate that internal-image anti-Ids that represent the configuration of the original antigen are rarely found (Kluskens and Köhler, 1974; Huang et al., 1988). However, anti-Ids that participate in the regulation of immune responses do arise. These types of anti-Id are termed regulatory anti-Ids, and in general they are not internal

images. Thus, it appears that internal-image anti-Ids do not represent a major portion of the immunoregulatory network. They are therefore not expected to be present in levels high enough to interfere with assay performance.

In conclusion, the immune network hypothesis offers a unique approach to transform epitope structures into Id determinants expressed on the surface of antibodies. According to this original network hypothesis, the Id–anti-Id interactions regulate the immune response of a host to a given antigen. Immunization with a given Ag will generate the production of antibodies against this Ag termed Ab1. This Ab1 can generate a series of anti-Id antibodies against Ab1 termed Ab2. Some of these Ab2 molecules can effectively mimic the three-dimensional structures of external Ags. These particular anti-Ids called Ab2β, which fit into the paratopes of Ab1, can induce specific immune responses similar to responses induced by nominal Ag. Anti-Id antibodies of the β type express the internal image of the Ag recognized by the Ab1 antibody and can be used as surrogate Ags. Immunization with Ab2β can lead to the generation of anti-anti-Id antibodies (Ab3) that recognize the corresponding original Ag identified by the Ab1. Several such Ab2β have been used in animal models to trigger the immune system to induce specific and protective immunity against bacterial, viral, and parasitic infections. The administration of Ab2β as surrogate tumor-associated Ags represents another potential application of the Id vaccine concept. Furthermore, the technical and commercial advantages of anti-Ids make them excellent alternatives to many conventional antigens for diagnostic immunoassays.

References

1. Amzel L.M., Garcia K.C., and Desiderio S., 1994. Do Anti-Idiotypic Antibodies Mimic Antigen? Research Immunol. 145:53-55.
2. Ardman B., Khiroya R.H., and Schwartz R.S., 1985. Recognition of a leukemia-related antigen by an antiidiotypic antiserum to an anti-gp70 monoclonal antibody. J. Exp. Med. 161:669-686.
3. Augustin A.A., Sim G.K., and Bona C.A., 1983. Internal images of antigens within the immune network. Surv. Immunol. Res. 2:78-87.
4. Ban N., Escobar C., and Garcia R., 1994. Crystal Structure of an Idiotype-Anti-Idiotype Fab Complex. Proc. Natl. Acad. Sci. USA, 91:1604-1608.
5. Beauclair K.D., and Khansari D.N., 1990. Protection of mice against Brucella abortus by immunization with polyclonal anti-idiotype antibodies. Immunobiology 180:208-220.
6. Benguric D.R., Dungu B., Thiaucourt F., and du Plessis D.H., 2001. Phage displayed peptides and anti-idiotype antibodies recognized by a monoclonal antibody directed against a diagnostic antigen of Mycoplasma capricolum subsp. capripneumoniae. Vet. Microbiol. 81:165-179.
7. Bentley G.A., Boulot G., Tiottot M.M., and Poljak R.J., 1990. Three-Dimensional Structure of an Idiotope-Anti-Idiotope Complex. Nature (London) 348:254-257.
8. Betakova T., Vareckova E., Kostolansky F., Mucha V., and Daniels R.S., 1998. Monoclonal anti-idiotypic antibodies mimicking the immunodominant epitope of

influenza virus haemagglutinin elicit biologically significant immune responses. J Gen. Virol. 79:461-470.

9. Bhattacharya-Chatterjee M., Chatterjee S.K., and Foon K.A., 2000. Anti-idiotype vaccine against cancer. Immunol. Lett. 74:51-58.
10. Binz H., Meier B., and Wigzell H., 1982. Induction or elimination of tumor-specific immunity against a chemically-induced rat tumor using auto-anti-idiotypic immunity. Int. J. Cancer 29:417-423
11. Bismuth G., Somme G., Roth C., Gougeon M.L., and Theze J., 1984. Poly (Glu60, Ala30, Tyr10) (GAT)-specific T cells do not express B cell public idiotopes but can be primed by monoclonal anti-idiotypic antibodies. Eur. J. Immunol. 14:503-510.
12. Bona C.A., 1989. Idiotype network theory and its implications in anti-tumor immunity. Prog. Clin. Biol. Res. 288:215-221.
13. Bona C.A., and Kohler H., 1984. Anti-idiotypic antibodies and internal images. In: Venter, J.C., Fraser, C.M., Linstrom, J. (Eds.), Monoclonal and Anti-idiotypic Antibodies: Probes for Receptor Structure and Function. Springer, New York, pp.141-149.
14. Burdette S., and Schwartz R.S., 1987. Current concepts: immunology. Idiotypes and idiotypic networks. N. Engl. J. Med. 317:219-224.
15. Casiglia D., Giardina, E. and Triolo, G., 1991. IgG auto-anti-idiotype antibodies against antibody to insulin in insulin-dependent (type 1) diabetes mellitus. Detection by capture enzyme linked immunosorbent assay (ELISA) and relationship with anti-insulin antibody levels. Diabetes Res. 16:181-184.

16. Chavez-Rueda K., Agundis-Mata C., Zenteno E., Shibayama M., Tsutsumi V., Munoz O., Leanos-Miranda A., and Blanco-Favela F., 2002. Development of a diagnostic test for Entamoeba histolytica using idiotype expression in human. J. Immunol. Methods 262:29-40.

17. Corbet S., Hirn M., Roth C., Theze J., Fougereau M., and Schiff C., 1988. Allogeneic manipulation of the GAT idiotypic cascade. Immunization of C57BL/6 mice by BALB/c anti-idiotypes stimulates similar strain-specific V genes as the original antigen. J. Immunol. 1; 141:779-784.

18. Couraud J.Y., Escher E., Regoli D., Imhoff V., Rossignol B., and Pradelles P., 1985. Anti-substance P anti-idiotypic antibodies. Characterization and biological activities. J. Biol. Chem. 260:9461-9469.

19. Couraud P.O., 1987. Anti-angiotensin II anti-idiotypic antibodies bind to angiotensin II receptor. J. Immunol. 138:1164-1168.

20. Dinca L., Neuwirth S., Schulman J., and Bona C., 1993. Induction of antihemagglutinin antibodies by polyclonal antiidiotype antibodies. Viral Immunol. 6:75-84.

21. Dreesman G.R., and Kennedy R.C., 1985. Anti-idiotypic antibodies: implications of internal image-based vaccines for infectious diseases. J. Infect. Dis. 151:761-765.

22. Eichmann K., and Rajewsky K., 1975. Induction of T and B cell immunity by antiidiotypic antibody. Eur. J. Immunol. 5:661.

23. Erlanger B.F., Cleveland W.L., Wassermann N.H., Ku H.H., Hill B.L., Sarangarajan R., Rajagopalan R., Cayanis E., Edelman I.S., and Penn A.S., 1986. Auto-anti-idiotype: a basis for autoimmunity and a strategy for anti-receptor antibodies. Immunol. Rev. 94:23-37.

24. Ertl H.C., Greene M.I., Noseworthy J.H., Fields B.N., Nepom J.T., Spriggs D.R., and Finberg R.W., 1982. Identification of idiotypic receptors on reovirus-specific cytolytic T cells. Proc. Natl. Acad. Sci. USA 79:7479-7483.

25. Farid N.R., Briones-Urbina R., and Nazrul-Islam M., 1982. Biologic activity of anti-thyrotropin anti-idiotypic antibody. Cell Biochem. 19:305-313.

26. Flood P.M., Kripke M.L., Rowley D.A., and Schreiber H., 1980. Suppression of tumor rejection by autologous anti-idiotypic immunity. Proc. Natl. Acad. Sci. USA 77:2209-2213.

27. Forstrom J.W., Nelson K.A., Nepom G.T., Hellstrom I., and Hellstrom K.E., 1983. Immunization to a syngeneic sarcoma by a monoclonal auto-anti-idiotypic antibody. Nature (London) 303:627-629

28. Francotte M., and Urbain J., 1984. Induction of anti-tobacco mosaic virus antibodies in mice by rabbit antiidiotypic antibodies. J. Exp. Med. 160:1485-1494.

29. Gaulton G., Co M.S., and Greene M.I., 1985. Anti-idiotypic antibody identifies the cellular receptor of reovirus type 3. J. Cell Biochem. 28:69-78.

30. Glasel J.A., 1989. Production and properties of antimorphine anti-idiotypic antibodies and their antiopiate receptor activity. Methods Enzymol. 178:222-243.

31. Goldbaum F.A., Velikovsky C.A., Dall'Acqua W., Fossati C.A., Fields B.A., Braden B.C., Poljak R.J., and Mariuzza R.A., 1997. Characterization of anti-anti-idiotypic antibodies that bind antigen and an anti-idiotype. Proc. Natl. Acad. Sci. USA 94:8697-8701.

32. Gramsch C., Schulz R., Kosin S., and Herz A., 1988. Monoclonal anti-idiotypic antibodies to opioid receptors. J. Biol. Chem. 263:5853-5859.

33. Greene M.I., and Nisonoff A., 1984. The Biology of Idiotypes, New York, Plenum pp, 299-313.

34. Grzych J.M., Capron M., Lambert P.H., Dissous C., Torres S., and Capron A., 1985. An anti-idiotype vaccine against experimental schistosomiasis. Nature (London) 316:74-76.

35. Haasemann M., Buschko J., Faussner A., Roscher A.A., Hoebeke J., Burch R.M., and Muller-Esterl W., 1991. Anti-idiotypic antibodies bearing the internal image of a bradykinin epitope. Production, characterization, and interaction with the kinin receptor. J. Immunol. 147:3882-3892.

36. Hayglass K.T., Benacerraf B., and Sy M.S., 1986. T cell development in B cell-deficient mice. V. Stopping anti-mu treatment results in Igh-restricted expansion of the T suppressor cell repertoire concomitant with the development of normal immunoglobulin levels. J. Exp. Med. 164:36-49

37. Hayunga E.G., Sumner M.P., Duncan Jr. J.F., Chakrabarti E.K., and Webert D.W., 1992. Production of anti-idiotypic antibodies as potential immunoreagents for the serological diagnosis of bovine cysticercosis. Tropical Vet. Med. 653: 178-183.

38. Herlyn D., Ross A.H., Iliopoulos D., and Koprowski H., 1987. Induction of specific immunity to human colon carcinoma by anti-idiotypic antibodies to monoclonal antibody CO17-1A. Eur. J. Immunol. 17:1649-1652.

39. Huang J.Y., Ward R.E., and Köhler H., 1988. Biological Mimicry of Antigenic Stimulation: Analysis of the in vivo antibody responses induced by monoclonal anti-idiotypic antibodies. Immunology 63:18.

40. Huang T., and Campadelli-Fiume G., 1996. Anti-idiotypic antibodies mimicking glycoprotein D of herpes simplex virus identify a cellular protein required for virus

spread from cell to cell and virus-induced polykaryocytosis. Proc. Natl. Acad. Sci. USA 93:1836-1840.

41. Irshad M., Gandhi B.M., Acharya S.K., Joshi Y.K., and Tandon B.N., 1987. An enzyme-linked immunosorbent assay (ELISA) for the detection of IgG and IgM anti-idiotypes directed against anti-HBs molecules. J. Immunol. Methods 96:211-217.
42. Jackson S., Mestecky J., Childers N.K., and Michalek S.M., 1990. Liposomes containing anti-idiotypic antibodies: an oral vaccine to induce protective secretory immune responses specific for pathogens of mucosal surfaces. Infect. Immun. 58:1932-1936.
43. Jerne N.K., 1974. Towards a network theory of the immune system. Ann. Immunol. (Paris). 125C:373-389.
44. Jerne N.K., Roland J., and Cazenva P.A., 1982. Recurrent idiotopes and internal images. EMBO J. 1:243-247.
45. Jiang Z., Zhou E-.M., Ameri-Mahabadi M., Zimmerman J.J., and Platt K.B., 2003. Identification and characterization of auto-anti-idiotypic antibodies specific for antibodies against porcine reproductive and respiratory syndrome virus envelope glycoprotein (GP5). Vet. Immunol. Immunopathol. 92:125-135.
46. Kato T., Takazoe I., and Okuda K., 1990. Protection of mice against the lethal toxicity of a lipopolysaccharide (LPS) by immunization with anti-idiotype antibody to a monoclonal antibody to lipid A from Eikenella corrodens LPS. Infect. Immun. 58:416-420
47. Kaufmann S.H., 1985. T cell clones and their products: experimental clues for the immunoprophylaxis and immunotherapy of intracellular bacterial infections? Infection 2:177-183.

48. Kearney J.F., Vakil M., and Solvason N., 1989. The role of idiotypic interactions and B-cell subsets in development of the B-cell repertoire. Cold Spring Harb. Symp. Quant. Biol. 54:203-207.

49. Kennedy R.C., and Dreesman G.R., 1983. Production and characterization of anti-idiotype reagents for the analysis of viral antigen systems. J. Virol. Meth. 7:103-115.

50. Kennedy R.C., and Dreesman G.R., 1984. Enhancement of the immune response to hepatitis B surface antigen. In vivo administration of antiidiotype induces anti-HBs that expresses a similar idiotype. J. Exp. Med. 159:655-665.

51. Kennedy R.C., Dreesman G.R., Butel J.S., and Lanford R.E., 1985. Suppression of in vivo tumor formation induced by simian virus 40-transformed cells in mice receiving antiidiotypic antibodies. J. Exp. Med. 161:1432-1449.

52. Kennedy R.C., Eichberg J.W., Lanford R.E., and Dreesman G.R., 1986. Anti-idiotypic antibody vaccine for type B hepatitis in chimpanzees. Science 232:220-223.

53. Kennedy R.C., and Dreesman G.R., 1986. Anti-idiotypic antibodies as idiotope vaccines that induce immunity against infectious igents. Intern. Rev. Immunol. 1:67-78.

54. Kim Y.T., Deblasio T., Thorbecke G.J., Weksler M.E., and Siskind G.W., 1989. Production of auto-anti-idiotype antibody during the normal immune response. XIV. Evidence for the antigen-independent operation of the idiotype network. Immunology 67:191-196.

55. Kleyman T.R., Kraehenbuhl J.P., and Ernst S.A., 1991. Characterization and cellular localization of the epithelial Na+ channel. Studies using an anti-Na+ channel antibody raised by an antiidiotypic route. J. Biol. Chem. 266:3907-3915.

56. Kluskens L., and Köhler H, 1974. Regulation of immune response by autogenous antibody against receptor. Proc. Natl. Acad. Sci. USA 71:5083.

57. Koprowski H., Herlyn D., Lubeck M., DeFreitas E., and Sears H.F., 1984. Human anti-idiotype antibodies in cancer patients: Is the modulation of the immune response beneficial for the patient? Proc. Natl. Acad. Sci. USA 81:216-219

58. Kresina T.F., and Olds G.R., 1989. Antiidiotypic antibody vaccine in murine *Schistosoma mansoni* comprising the internal image of antigen. J. Clin. Invest. 83:912-920.

59. Kunkel H.G., Mannik M., and William R.C., 1963. Individual Antigenic Specificities of Isolated Antibodies. Science 140:1218-1219.

60. Langone J.J., 1989. Methods in Enzymology. Volume 178, Antibodies, Antigens, and Molecular Mimicry, San Diego, Academic Press.

61. Lee V.K., Hellstrom K.E., and Nepom G.T., 1986. Idiotypic interactions in immune responses to tumor-associated antigens. Biochim. Biophys. Acta 865:127-139

62. Lin M., and Zhou E.-M., 1995. Internal image rabbit anti-idiotypic antibody detects sheep antibodies to the bluetongue virus core protein VP7. Immunotechnology 1:151-155.

63. Linthicum D.S., Kussie P.H., Combs S., and Yatko D., 1988. Idiotypes and anti-idiotypes: significance in immunoassays. J. Clin. Immunol. 11:31-36.

64. Linthicum D.S., Bolger M.B., Kussie P.H., Albright G.M., Linton T.A., Combs S., and Marchetti D., 1988. Analysis of idiotypic and anti-idiotypic antibodies as models of receptor and ligand. Clin. Chem. 34:1676-1680.

65. McNamara M.K., Ward R.E., and Kohler H., 1984. Monoclonal idiotope vaccine against Streptococcus pneumoniae infection. Science 226:1325-1326.

66. Mittelman A., Chen Z.J., Yang H., Wong G.Y., and Ferrone S., 1992. Human high molecular weight melanoma-associated antigen (HMW-MAA) mimicry by mouse anti-idiotypic monoclonal antibody MK2-23: induction of humoral anti-HMW-MAA immunity and prolongation of survival in patients with stage IV melanoma. Proc. Natl. Acad. Sci. USA 89:466-470.

67. Mosier D.E., and Feeney A.J., 1984. The Biology of Idiotypes; Greene, M.I., and Nisonoff A., eds., Plenum Press, New York, pp. 403-416

68. Nelson K., and Nepom G.T., 1986. Paradoxes in Immunology, Hoffman, G., Levy, J.G., and Nepom, G.T. eds., CRC Press, Boca Raton, Florida, pp. 177-185.

69. Nepom G.T., Tardieu M., Epstein R.L., Noseworthy J.H., Weiner H.L., Gentsch J., Fields B.N., and Greene M.I., 1982. Virus-binding receptors: similarities to immune receptors as determined by anti-idiotypic antibodies. Surv. Immunol. Res. 1:255-261.

70. Nepom G.T., Hellstrom I., and Hellstrom K.E., 1983. Suppressor mechanisms in tumor immunity. Experientia 39:235-242.

71. Nepom G.T., Nelson K.A., Holbeck S.L., Hellstrom I., and Hellstrom K.E., 1984. Induction of immunity to a human tumor marker by in vivo administration of anti-idiotypic antibodies in mice. Proc. Natl. Acad. Sci. USA 81:2864-2867.

72. Nisonoff A., and Lamoyi E., 1981. Implications of the presence of an internal image of the antigen in anti-idiotypic antibodies: possible application to vaccine production. Clin. Immunol. Immunopathol. 21:397

73. Nisonoff A., 1991. Idiotypes: concepts and applications. J. Immunol. 147:2429-2438.

74. Oosterlaken T.A., Brandenburg A., Schielen P., Fransen R., Kraaijeveld C.A., and Snippe H., 1991. Efficient induction of Semliki Forest virus and mumps virus neutralizing anti-anti-idiotypic antibodies using Quil A as adjuvant. J. Immunol. Methods 136:169-175.

75. Orten D.J., Reddy P.G., Reddy D.N., Xue W., Abdelmagid O.Y., Blecha F., and Minocha H.C., 1991. Induction of immune response to bovine herpesvirus-1 with anti-idiotypic antibodies. Viral Immunol. 4:111-122.

76. Oudin J., and Michel M., 1969. Idiotypy of rabbit antibodies. I. Comparison of idiotypy of antibodies against Salmonella typhi with that of antibodies against other bacteria in the same rabbits or of antibodies against Salmonella typhi in various rabbits. J. Exp. Med. 130:595-617.

77. Pain D., Murakami H., and Blobel G., 1990. Identification of a receptor for protein import into mitochondria. Nature (London) 347: 444-449

78. Percy A.J., and Harn D.A., 1988. Monoclonal anti-idiotypic and anti-anti-idiotypic antibodies from mice immunized with a protective monoclonal antibody against Schistosoma mansoni. J. Immunol. 140:2760-2762.

79. Potocnjak P., Zavala, F., Nussenzweig, R., and Nussenzweig, V., 1982. Inhibition of idiotype-anti-idiotype interaction for detection of a parasite antigen: a new immunoassay. Science 215:1637-1639.

80. Reis D.D., Gazzinelli R.T., Gazzinelli G., and Colley D.G., 1993. Antibodies to Trypanosoma cruzi express idiotypic patterns that can differentiate between patients with asymptomatic or severe Chagas' disease. J. Immunol. 150:1611-1618.

81. Rodkey L.S., 1980. Autoregulation of immune responses via idiotype network interactions. Microbiol. Rev. 44:631-659.

82. Sacks D.L., and Sher A., 1983. Evidence that anti-idiotype induced immunity to experimental African trypanosomiasis is genetically restricted and requires recognition of combining site-related idiotopes. J. Immunol. 131:1511-1515.

83. Sacks D.L., Kirchhoff L.V., Hieny S., and Sher A., 1985. Molecular mimicry of a carbohydrate epitope on a major surface glycoprotein of Trypanosoma cruzi by using anti-idiotypic antibodies. J. Immunol. 135:4155-4159.

84. Schick M.R., Dreesman G.R., and Kennedy R.C., 1987. Induction of an anti-hepatitis B surface antigen response in mice by noninternal image (Ab2 alpha) anti-idiotypic antibodies. J. Immunol. 138:3419-3425.

85. Sege K., and Peterson P.A., 1978. Use of Anti-Idiotypic Antibodies as Cell Surface Receptor Probes. Proc. Natl. Acad. Sci. USA 75: 2443-2447.

86. Su S., Ward M.M., Apicella M.A., and Ward R.E., 1992. A nontoxic, idiotype vaccine against gram-negative bacterial infections. J. Immunol. 148:234-238.

87. Tackaberry E.S., Hamel J., Larose Y., and Brodeur B.R., 1993. Anti-idiotypic mimicry of a neutralizing epitope on the glycoprotein B complex of human cytomegalovirus. J. Virol. 67:6815-6819.

88. Thanavala Y.M., Brown S.E., Howard C.R., Roitt I.M., and Steward M.W., 1986. A surrogate hepatitis B virus antigenic epitope represented by a synthetic peptide and an internal image antiidiotype antibody. J. Exp. Med. 164:227-236.

89. Thanavala Y.M., and Roitt I.M., 1986. Monoclonal anti-idiotypic antibodies as surrogates for hepatitis B surface antigen. Int. Rev. Immunol. 1:27-39.

90. Thomas W.R., Morahan G., and Miller J.F., 1983. Induction of suppressor T cells by monoclonal anti-idiotope antibody in strains of mice not expressing the idiotope in hyperimmune serum. J. Immunol. 130:2079-2082.

91. Tilkin A.F., Schaaf-Lafontaine N., Van Acker A., Boccadoro M., and Urbain J., 1981. Reduced tumor growth after low-dose irradiation or immunization against blastic suppressor T cells. Proc. Natl. Acad. Sci. USA 78:1809-1812.

92. Urbain J., Slaoui M., and Leo O., 1982. Idiotypes, recurrent idiotypes and internal images. Ann. Immunol. (Paris). 133D:179-189

93. Vaux D., Tooze J., and Fuller S., 1990. Identification by anti-idiotype antibodies of an intracellular membrane protein that recognizes a mammalian endoplasmic reticulum retention signal. Nature (London) 345:495-502.

94. Velge-Roussel F., Verwaerde C., Grzych J.M., Auriault A., and Capron A., 1989. Protective effects of anti-antiidiotypic IgE antibodies obtained from an IgE monoclonal antibody specific for a 26-kilodalton Schistosoma mansoni antigen. J. Immunol. 142:2527-2532.

95. Victor-Kobrin C., Barak Z., Rothe J., Rubinstein L.J., and Bona C.A., 1989. The role of the A48 regulatory idiotype in inducing tumor-specific immunity. Int. Rev. Immunol. 4:321-336.

96. Vogel R., Kaufmann J., Chung D.W., Kellermann J., and Muller-Esterl W., 1990. Mapping of the prekallikrein-binding site of human H-kininogen by ligand screening of lambda gt11 expression libraries. Mimicking of the predicted binding site by anti-idiotypic antibodies. J. Biol. Chem. 265:12494-12502.

97. Walter G., Friesen H.J., and Harthus H.P., 1988. Anti-idiotypic antibodies: powerful tools in diagnosis and therapy. Behring Inst. Mitt. 82:182-192.

98. Williams W.V., Guy H.R., and Weiner D.B., 1989. Three-dimensional structure of a functional internal image. Viral. Immunol. 2:239-246.

99. Yongjuan X., Weiquan H., Baocheng H., Xiaohang J., and Rongqing Z., 2002. Production and characterisation of monoclonal anti-idiotype antibody to Vibrio anguillarum. Fish Shellfish Immunol. 12:273-281.

100. Zhou E.-M., Chanh T.C., Dreesman G.R., Kanda P., and Kennedy R.C., 1987. Immune response to human immunodeficiency virus: in vivo administration of anti-idiotype induces an anti-gp 160 response specific for a synthetic peptide. J. Immunol. 139:2950-2956.

101. Zhou E.-M., Dreesman G.R., and Kennedy R.C., 1987. Anti-idiotypic antibodies: a new generation of vaccines against infectious agents. Microbiological Sci. 4:36-40.

102. Zhou E.-M., Lohman K.L., and Kennedy R.C., 1990. Administration of noninternal image monoclonal anti-idiotypic antibodies induces idiotype-restricted responses specific for human immunodeficiency virus envelope glycoprotein epitopes. Virology 174:9-17.

103. Zhou E.-M., Dzuba-Fischer J.M.M., Rector E.S., Sehon A.H., and Kisil F.T., 1991. A murine monoclonal anti-idiotypic antibody detects a common idiotype on human, mouse and rabbit antibodies to allergen Lol pIV. Scand. J. Immunol. 34:307-316.

104. Zhou E.-M., Afshar A., Heckert R.A., and Nielsen K., 1994. Anti-idiotypic antibodies generated by sequential immunization detect shared idiotype on antibodies to pseudorabies virus antigens. J. Virol. Meth. 48:301-313.

105. Zhou E.-M., and Huang W., 1995. Anti-idiotypic antibody as potential serodiagnostic reagent for detection of bluetongue virus infection. J. Clin. Microbiol. 33:850-854.

106. Zhou E-M., and Kisil F.T., 1995. Regulation of levels of serum antibodies to ryegrass pollen allergen Lol pIV by an internal image anti-idiotypic monoclonal antibody. Immunology 84:343-349.

107. Zhou E.-M., and Lin M., 1997. Idiotypes in Medicine: Autoimmunity, Infection and Cancer; Shoenfeld, Y., Kennedy, R.C., and Ferrone, S. eds., Idiotypes in Medicine: Autoimmunity, Infection and Cancer, Elsevier, Amsterdam, The Netherlands, pp. 347-355.

108. Zhou E-M., 1999. Anti-idiotype technique: an alternative approach for immunodiagnosis of bluetongue. Vet. Immunol. Immunopathol. 72:237-242.

109. Zhou E-.M., Clavijo A., Jiang Z., Ameri-Mahabadi M., and Zimmerman J.J., 2004. Induction of auto-anti-idiotypic antibodies specific for antibodies to matrix and envelope glycoplrotein from pigs experimentally infected with porcine reproductive and respiratory syndrome virus. Vet. Immunol. Immunopathol. 101:49-59.

CHAPTER 2. Monoclonal Anti-Idiotypic Antibody Specific for Antibodies Against GP5 Antigen of Porcine Reproductive and Respiratory Syndrome Virus Elicits Specific Immune Response in Mice

A paper to be submitted to Viral Immunology

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Abstract

A syngeneic mouse monoclonal anti-idiotypic antibody (anti-Id), designated M8G, was generated against a monoclonal antibody (MAb 25C) specific for porcine reproductive and respiratory syndrome virus PRRSV glycoprotein 5 (GP5). Observations that (i) the anti-Id inhibited the binding of MAb 25C to PRRSV antigen, and (ii) the Id-anti-Id interaction could be inhibited by PRRSV antigen indicated that the Id was located within or near the antigen combining site. These properties served to characterize M8G as an internal image anti-Id. Evidence that an immune response in pigs experimentally infected with PRRSV elicits the formation of antibodies that express a common Id was provided by the observation that the Id-anti-Id interactions could be inhibited by pig antisera to PRRSV antigen. The anti-anti-Id

antibodies (Ab3) induced with monocolonal anti-Id (MAb2) competed with MAb1 binding to PRRSV antigen. Furthermore, the binding of anti-anti-Id to MAb2 was inhibited with PRRSV antigen, indicating that anti-anti-Id mimics the structure of the epitope in GP5 that was recognized with MAb1. These serologic findings suggest that M8G carries the internal image of the antigen.

Introduction

Porcine reproductive and respiratory syndrome (PRRS), caused by the PRRS virus (PRRSV), is currently among the most significant pathogens of swine worldwide. PRRSV is a member of the family *Arteriviridae* in the order *Nidovirales* (6). PRRSV is an enveloped RNA virus, and has a 15-kb positive-sense, single-stranded RNA genome. PRRSV encodes an approximately 4,000-amino-acid large replicase polyprotein (open reading frame [ORF] 1a and 1b) and six structural proteins of 130 to 265 amino acids (ORFs 2 to 7) (9,32). Three of these structural proteins have been identified as envelope (25 kDa), matrix (19 kDa), and nucleocapsid (15 kDa) proteins, i.e. GP5, M, and N proteins, respectively (1,2,22). Three additional proteins with molecular masses of 29–30 kDa (GP2), 45–50 kDa (GP3), and 31–35 kDa (GP4) have been identified in purified Lelystad virus and are presumed to be associated with the viral envelope (10,22,23,36). Among these viral proteins, GP5 is the major envelope protein and serum neutralizing antibody titers were significantly correlated with anti-GP5 titers (29,31,35,37). The humoral immune response is presumed to play an important role in resistance to reinfection and in prevention or reduction of viral spread from animal to animal, since neutralizing antibodies have the potential to clear free virus from the circulation (25).

Anti-PRRSV immunoglobulins in serum after PRRSV infection can be detected within 7 days of infection. (20,25,26,30,38). Neutralizing antibodies to PRRSV develop between 1 and 2 months following exposure (11,20,24,26,38). Anti-PRRSV antibodies may persist in blood for the lifetime of commercial pigs (25), and PRRSV can persist in some animals despite high levels of antiviral antibodies which is associated with long-term, intermittent shedding or seminal excretion of the virus (7,13).

An idiotope is a single antigenic determinant that is located on the variable regions (V domain) of both antibody molecules and receptor molecules of T and B lymphocytes. Idiotopes may be either in the antigen binding site or on the framework regions of the variable domain. The sum of the idiotopes on an antibody's V domain determines its idiotype. The idiotype (Id) network theory of immune regulation offered by Jerne (15), propose that the immune response to a given antigen can be regulated by a series of Id and its serological counterpart, an anti-idiotypic antibody (anti-Id or Ab2), has been found useful for the production of diagnostic reagents and vaccines (19). Upon immunization with an idiotypic antibody (Ab1), the corresponding anti-Ids are elicited and can be serologically classified into four categories (Ab2α, Ab2β, Ab2γ and Ab2ε) (4,16). Ab2α recognizes an Id associated with the framework region of Ab1. Ab2β, an internal image anti-Id Ab2, recognizes an Id within the antigen-combining site and bears a structural resemblance to the original antigen. The anti-Id mimicry of the original antigen has been experimentally proved in many virus systems (19,28,39). Ab2γ, like Ab2β, recognizes an Id within the antigen-combining site but does not carry the internal image of the original antigen. Ab2ε is defined as the antibody that binds both Ab1 and antigen (4). A given idiotypic antibody is under the

control of an anti-Id, whereas the anti-Id can be regulated by anti-anti-Id (or Ab3). The concept of internal image stems from the possibility of raising Ab3 antibodies that would recognize the original Ag. If one takes into account both the specificity and the idiotypic characteristics of Ab1 molecules, four discrete types of Ab3 molecules may be anticipated: Id^-Ag^-, Id^+Ag^-, Id^-Ag^+ and Id^+Ag^+, the later being very similar to Ab1 and termed Ab1' (8).

PRRSV infection poses a challenge to current serodiagnostic and vaccination strategies. Because the genetic diversity of field PRRSV isolates is very high, vaccine efficacy against heterologous challenge may be limited (34). Also, live PRRSV vaccines have been observed to revert to virulence (5,33), and the safer, killed vaccines have so far proved less effective (27). A better understanding of the mechanisms that regulate immunity to this virus will aid in the development of more effective vaccines.

In this study, we describe the generation and characterization of a monoclonal anti-Id produced against a mouse MAb specific for GP5 of PRRSV and determined the structure of Ab3 (Id^+Ag^+ or Id^+Ag^-). The monoclonal anti-Id was classified as Ab2β preparation. Immunization with Ab2β induced an anti-GP5 response. Serologically, the anti-anti-Id antibodies (Ab3) induced with anti-Id MAb2 competed with Ab1 binding to PRRSV antigen, inhibited the Id-anti-Id reaction, and expressed a similar Id present on the mouse monoclonal Ab1. These results indicate that an Id-anti-Id network may be operational in modulating the immune response to GP5 in PRRSV infection.

Materials and Methods

PRRSV antigen. A cell line, MARC-145 was infected with VR-2332 of PRRSV isolate at a multiplicity of infection of approximately 5 and the infected cell culture was then incubated and harvested as described by Yoon et al. (38). The total protein concentration of the crude cell culture antigen preparation was determined by the method of Lowry et al. (21) using a protein assay kit (Pierce, Rockford, IL).

Preparation of MAb1. Hybridoma cells secreting MAb1 specific for the PRRSV glycoprotein (GP5), which is denoted as 25C (IgG_1, λ), was originally developed by Yang et al. (37). The 25C-IgG was purified from supernatant of hybridoma culture using ammonium sulfate precipitation method (14). IgG concentration was estimated at 280 nm using a spectrophotometer (SmartSpec 3000, Bio-Rad Laboratories, Hercules CA) with the extinction coefficient of 1.35. The purity of antibodies was confirmed by SDS-PAGE.

Enzymatic digestion of the MAb1. To prepare $F(ab')_2$, the purified 25C-IgG was digested using pepsin (Sigma, St. Louis, MO) as described previously (17,41). The $F(ab')_2$ fragments were then purified by the Protein A affinity column to remove Fc fragments. SDS-PAGE and ELISA were used to confirm purity of $F(ab')_2$.

Pig sera. Serum samples were collected from a group of pigs experimentally infected with PRRSV. The presence of anti-PRRSV antibodies was confirmed by IFA and HerdCheck ELISA as described previously (17,41).

Production of MAb2 specific for GP5 of PRRSV. Six week-old female BALB/c mice, were immunized intraperitoneally (IP) with 25C-F(ab')$_2$-KLH (50 μg in 100 μl of 0.01 M PBS) mixed with Complete Freund's Adjuvant (Sigma, St. Louis, MO) followed by two injections of 25C-F(ab')$_2$-KLH (50 μg in 100 μl of PBS) mixed with Incomplete Freund's Adjuvant (Sigma, St. Louis, MO) on day 14 and 28. Blood was taken from each mouse and the antibody response was measured by ELISA (as described below). The mouse with the highest antibody titer in its serum was selected as the spleen donor and was given an intravenous booster injection of 0.1 ml of 25C-F(ab')$_2$-KLH in PBS, 3 days before fusion. The spleen cells from the immunized mouse were fused with SP2/O myeloma cells, as described elsewhere (18). Hybridoma culture supernatants were examined for the presence of antibody by using 25C-F(ab')$_2$ fragments at 2 μg/ml (100 μl/well) in sodium phosphate buffer (PBS; 10 mM, pH 7.2) in an ELISA format. Hybridomas that reacted positively were cloned by the limiting dilution method. A monoclonal anti-Id, designated M8G, was identified as an IgG1 (κ) using the mouse IsoStrip kit (Boehringer Mannheim, Indianapolis, IN). IgG concentration was estimated at 280 nm using a spectrophotometer (SmartSpec 3000, Bio-Rad Laboratories, Hercules CA) with the extinction coefficient of 1.35. The purity of antibodies was confirmed by SDS-PAGE.

Labelling Antibodies. Conjugation of MAb1 (25C) to Biotin and MAb2 (M8G) to Horseradish Peroxidase (HRP) were performed based on the documentation protocol by the manufacturer (Pierce, Rockford, IL). Optimum dilutions of the conjugates were determined using ELISA and Western blot assay.

Enzymatic digestion of the MAb2. MAb2-F(ab')$_2$ was prepared based on the protocol described for making MAb1-F(ab')$_2$. A papain digestion was performed to prepare MAb2-Fab using protocol described elsewhere (14). The Fab fragments were purified using a Protein A affinity column and Fc fragments were removed. The purity of Fab was confirmed using SDS-PAGE and ELISA.

Production of anti-anti-idiotypic antibody (Ab3) specific for GP5 of PRRSV. For induction of Ab3, MAb2 was used in the form of Fab coupled to KLH (Fab-KLH). Three, six week-old female BALB/c mice were immunized intraperitoneally on day 0, with 50 μg of Mab$_2$-Fab-KLH (50 μg in 100 μl of PBS) emulsified 1:1 in Freund's complete adjuvant (Total vol. = 0.2 ml). Two booster injections were given on days 14 and 28 each with 50 μg of the same anti-Id preparation in Freund's incomplete adjuvant. Two weeks after the final injection, mice were bled and serum samples tested for the presence of anti-anti idiotypic antibody (Ab3) in serum by an ELISA with MAb2-Fab on solid-phase. The antibody end point titre (EPT) was expressed as the highest serum dilution that gave the absorbance at 490 nm 3-fold higher than that for pre-immune serum.

Characterization of monoclonal anti-idiotypic antibodies specific for GP5

a) Indirect ELISA. An indirect ELISA was used to detect the direct binding between MAb2-M8G and MAb1-25C. The solid phase was prepared by coating the wells of ELISA plates with 25C-F(ab')$_2$ at 2 μg/ml (100 μl per well) in PBS (0.01 M, pH 7.2) at 4°C overnight. As a control, normal mouse F(ab')$_2$ (Pierce, Rockford, IL) and two other monoclonal antibodies against matrix and nucleoprotein of PRRSV designated as 19Bd and

15E, respectively were similarly used. After removal of excess antibodies by washing the plate with PBS containing 0.05 % of Tween-20 (PBS-T, v/v) three times, purified M8G at different concentrations was added to the wells (100 µ1 per well) and incubated for 45 min at room temperature. The wells were then washed three times with PBS-T. The MAb2 that had bound to the MAbl was detected by adding HRP-goat anti-mouse Fcγ (Jackson ImmunoResearch, West Grove, PA) at 1:2000 dilution in PBS-T to each well and the plate was incubated for 45 min at room temperature. After washing the wells with PBS-T, the substrate *o*-phenylene diamine (OPD, 100 µl/well; Sigma, St. Louis, MO) was used to develop the calorimetric reaction. Finally, stop solution (H_2SO_4, 3 M) was added to each well at 100 µl per well. The optical density (OD) at 490 nm of each well was measured on an automatic ELISA plate reader (Universal Microplate Reader, EL800, Bio-Tek Instrument, Inc., Winooski, VT). The assay was performed in duplicate.

b) Western blot analysis. To further confirm the specificity of M8G for 25C Western blot immunoassays was performed based on a previously developed method (40) with the following modification: 5 µg/lane of 25C-IgG or normal mouse IgG (as a control) were loaded on the SDS-PAGE under both reducing and non-reducing conditions and then transferred to PVDF membrane (Bio-Rad Laboratories, Hercules, CA). The membrane was blocked with 1% BSA (w/v) in PBS-T at room temperature for 1 h and incubated with HRP-M8G at 1:1000 dilution for another hour. The membrane was washed three times with PBS-T and then two times for 5 minutes with PBS (pH 7.2) to remove the detergent. Finally, 3,3'-diaminobenzidine tetra hydrochloride (DAB) liquid substrate system (Sigma, St. Louis, MO) was added to develop the color reaction.

c) Competitive ELISAs. To determine whether swine antisera (Ab1) could inhibit MAb1 binding to MAb2 a competitive ELISA (C-ELISA) was employed. The solid phase was prepared by coating the wells of microtiter plates (Nalge Nunc International, Roskilde, Denmark) with M8G-F(ab')$_2$ (0.2 µg/well). The biotinylated 25C (1/10000 dilution) was used in the liquid-phase with or without mixing with individual competitors including swine serum recovered from pigs that had been experimentally infected with PRRSV (at 1/10 and 1/100 dilution). The conjugate used in the C-ELISA was HRP-Streptavidin (Jackson ImmunoResearch, West Grove, PA) at 1:2000 dilution. The addition of the substrate and quantitation of the calorimetric reaction was performed as described above. The percent inhibition was calculated by the following formula:

% INHIBITION = [1 - (OD_{490} with competitor - background OD_{490}) / (OD_{490} without competitor - background OD_{490})] × 100.

To determine whether PRRSV antigen could inhibit MAb1 binding to MAb2, a C-ELISA was performed basically the same as described above, except that purified 25C- F(ab')$_2$ (0.2 µg/well) was used to coat the ELISA plate. M8G-HRP (at a final concentration of 1:2000) was used in the liquid-phase with or without mixing with individual competitors including PRRSV antigen and MARK 145 cell line as a control at 1000, 100 and 10 µg/ml. the rest of assay was performed as described above.

d) IFA. To ascertain whether the anti-Id could bind to antibody against GP5 and inhibit its binding to PRRSV, an indirect immunofluorescence assay (IFA) was employed. The IFA performed was based on the procedure described elsewhere (Yoon et al., 1995). In brief,

MARC-145 cells cultured on slides were infected with VR-2332 strain of PRRSV. After culturing for 2 days, the infected cells were fixed and probed directly with MAb against GP5 (25C) (4 μg/ml) or premixed with M8G (at different concentrations). Normal mouse IgG was used as a control. Binding of 25C to PRRSV was visualized using goat anti-mouse IgG coupled with fluorescein isothiocyanate (FITC, 1/50 dilution; Jackson ImmunoResearch, West Grove, PA).

Characterization of anti-anti-idiotypic (Ab3s) antibodies specific for GP5

a) Detection of Ab3. An I-ELISA was used to determine the specificity of the binding of serum Ab3 antibodies to MAb2 (M8G). The solid phase was prepared by coating the wells of ELISA plates with M8G-F(ab')$_2$ fragments at 2 μg/ml (100 μl/well) in PBS (10 mM, pH 7.2) at 4 °C overnight. After washing the plate with PBS-T, individual mouse serum diluted 1:100, 1:1000, or 1:10000 in PBS-T were added to duplicate wells (100 μl/well) and incubated for 45 min at room temperature. The wells were, then washed three times with PBS-T and the presence of Ab3 that bound to the solid-phase M8G-F(ab')$_2$ was detected by the addition of HRP-goat anti-mouse Fcγ diluted 2000-fold in PBS-T (100 μl/well) for 45 min at room temperature. After final washing with PBS-T for three times, substrate solution (OPD, 100 μl/well) was added to all wells to develop the colorimetric reaction at the room temperature for 15 min. The reaction was then stopped by adding 3 M H2SO4 to each well (100 μl/well) and read at OD 490 nm on an automatic ELISA plate reader.

b) Competitive ELISAs. A C-ELISA was performed to determine whether Ab3 could inhibit MAb1 binding to MAb2. For this purpose, the solid phase was prepared by coating

the wells of ELISA plate with M8G-F(ab')$_2$ (0.2 μg/well). The Biotin-25C (1/10000 dilution) was used in the liquid-phase with or without mixing with individual mice sera immunized with M8G, at 1/10 and 1/100 dilution. A hundered microliter of the mixture were added to the wells and kept for 1 h at room temperature followed by washing three times with PBS-T. The conjugate used in the C-ELISA was HRP-Streptavidin (Jackson ImmunoResearch, West Grove, PA) at 1:2000 dilution in PBS-T (100 μl/well). The rest of assay was performed as the same described previously.

To determine whether PRRSV antigen could inhibit MAb2 binding to Ab3, a C-ELISA was performed basically the same as described above, except that individual purified M8G-F(ab')$_2$ (0.2 μg/well) was used to coat the ELISA plate. The mouse serum (M1, at the final concentration of 1:1000) was used in the liquid-phase with or without mixing with individual inhibitors including PRRSV antigen and MARK 145 cell line as a control at 1000 and 100 μg/ml. After washing the plates 3 times with PBS-T, HRP-goat anti-mouse Fcγ (Jackson ImmunoResearch, West Grove, PA) diluted 2000-fold in PBS-T (100 μl/well) for 45 min at room temperature was used. The addition of the substrate and quantitation of the calorimetric reaction was performed as described above.

To further confirm the specificity of Ab3 for PRRSV, the ability of Ab3 to inhibit Ab1 from binding to the solid-phase PRRSV antigen was tested by competitive ELISA. For this purpose 96-well microtiter plates were coated with PRRSV Ag (100 μl/well) at 2 μg/ml in PBS (0.01M, pH 7.2) and incubated over night at 4°C. The Biotin-25C (1/10000 dilution) was used in the liquid-phase with or without mixing with individual mouse sera at 1/10 and 1/100 dilution. Pre-immune serum was used as a control. A hundered microliter of the

mixture was added to the wells and kept for 1 h at room temperature followed by washing three times with PBS-T. The conjugate used in the C-ELISA was HRP-Streptavidin (Jackson ImmunoResearch, West Grove, PA) at 1:2000 dilution in PBS-T (100 µl/well). These values were compared with the maximum binding of the Ab_1, which occurred in the absence of a competitor. The percent inhibition was calculated using the formula described above.

Results

Characterization of monoclonal anti-idiotypic antibody specific for GP5

Specificity of the monoclonal anti-Id M8G. The specificity of the mouse monoclonal anti-Id M8G was tested by two different assays. First, an I-ELISA was employed (Fig.1). The lowest concentration of M8G that bound to solid-phase 25C was 1.56 µg/ml. Monoclonal anti-Id did not bind to other $F(ab')_2$ fragments of monoclonal antibodies directed to PRRS virus M protein (19Bd) or nucleoprotein (15E) or to normal mouse $F(ab')_2$.

In addition, Western blot analysis was employed to confirm the immunoreaction between Mab1 and Mab2. As shown in Fig.2, membrane-bound 25C reacted with M8G. It should be noted that monoclonal anti-Id did not react with membrane-bound normal mouse IgG. The results of Western blot showed that the monoclonal anti-Id recognizes the idiotype associated with both heavy and light chains of the MAb1 (Fig.2).

Monoclonal anti-Id recognizes an Id common to antibodies to GP5 of PRRSV. The ability of pig antisera to inhibit 25C from binding to monoclonal anti-Id was determined using a C-ELISA. As shown in Table 1, antisera from pigs experimentally infected with

PRRSV, inhibited 25C from binding to the solid-phase M8G. The degree of inhibition with antisera from pigs experimentally infected with PRRSV was 20.4 ± 1.1 to 29.5 ± 0.8, which may simply be because these pigs infected with whole virus were not expected to produce high titer antibodies against GP5 of PRRSV as detected by SN (data not shown).

Monoclonal anti-Id possesses the characteristics of an internal image anti-Id. The anti-Id M8G was examined to determine if it possessed the properties of an internal image anti-Id, i.e. whether or not: (i) the binding of 8MG to 25C could be inhibited by PRRSV antigen and (ii) anti-Id M8G could inhibit the binding of 25C to PRRSV antigen.

A C-ELISA was performed to find out whether PRRSV antigen could inhibit 25C binding to M8G. As shown in Fig. 4, the degrees of inhibition were found to be antigen concentration dependent. In the presence of PRRSV antigen at the final concentration of 500 μg/ml, the binding of anti-Id M8G to solid phase 25C was inhibited by 83%, whereas 22.6% inhibition was achieved with MARK 145 cell line at the same concentration (Fig.3).

An IFA was employed to determine whether MAb2 could inhibit MAb1 binding to PRRSV. In the presence of M8G (100 μg/ml), the binding of 25C to PRRSV was inhibited to the extent of 100% (Table 2).

Together, these results indicated that M8G possesses the characteristics of an internal image anti-Id which recognizes the Id located within or near the antigen combining sites of the antibody to GP5.

Characterization of anti-anti-idiotypic antibody (Ab3) specific for GP5

Induction of anti-anti-Id antibody. Immunization with M8G in a syngenic system was done and the induced anti-anti-Id antisera (Ab3) were analyzed with an indirect ELISA. Fig.4 shows the binding activities of three mouse serum immunized with M8G.

Ab3 antibodies recognize the Ab1-specific epitope of GP5. To determine whether Ab3 possessed Ab1-like (Ab1') properties with specificity for the PRRSV antigen, two competitive ELISA were performed. First a C-ELISA was used to determine whether PRRSV antigen could inhibit MAb2 binding to Ab3. As shown in Fig. 5, PRRSV antigen compare to MARK 145 cell line, significantly ($P<0.05$) inhibited (73 and 53 % at 1000 and 100 μg/ml, respectively) Ab3 binding to M8G. To further confirm the specificity of Ab3 for PRRSV antigen, the immune sera were tested additionally by the second immunoassay. A C-ELISA was employed for the ability of immune serum (M1) to inhibit Ab1 (25C) from binding to the solid-phase PRRSV antigen. As shown in Fig. 6 the mouse serum compare to pre-immune sera significantly ($P<0.05$) inhibited the 25C-PRRSV interaction (at a dilution of 1:10).

Ab3 possess the Id of Ab1. To determine whether Ab3 expressed an Id of Ab1, the immune sera were evaluated for their ability to inhibit 25C from binding to the solid-phase M8G-$F(ab')_2$ by a C-ELISA. As Table 3 shows, the percent of inhibition of Id-anti-Id reaction by immune sera ranged from 30 ± 1.5 to 86 ± 1.1. Pre-immune serum did not affect binding of 25C to M8G.

The results show that murine Ab3 antibody recognized the same or a closely related epitope that is recognized by 25C. Also theses results confirm the fact that the M8G, carry the internal image of GP5.

Discussion

This report describes the generation and characterization of a monoclonal anti-Id, designated as M8G, directed to an Id on monoclonal antibody, 25C. 25C recognized an epitope referred to as GP5 on the PRRSV (37). The finding that Id (25C)-anti-Id (M8G) interaction could be inhibited by swine antisera to PRRSV (Table 1) indicated that the Id is common to antibodies that are formed to GP5 in unrelated species. Moreover, evidence was also obtained which revealed that the interaction between the Id-anti-Id could be inhibited by PRRSV antigen (Fig.3). These observations, along with the fact that monoclonal anti-Id inhibited the interaction between MAb1 and PRRSV antigen (Table 2), indicate that M8G recognized an Id either within or near the antigen combining sites of 25C. According to the classification of anti-idiotypic antibodies (4), M8G has the characteristics of an internal image anti-Id. Since the antibodies to GP5 in the swine sera are of polyclonal origin and therefore are capable of recognizing any of the determinants of PRRSV e.g. M and N proteins and or other glycoproteins (GP2, GP3, GP4), it is evident that only that fraction of the entire repertoire of antibodies which is (i) directed to GP5 of PRRSV and (ii) possesses the Id which is the same or similar to that 25C, has the capacity to inhibit the Id-anti-Id interaction. Therefore, it was not surprising to find that the swine sera were not able to completely inhibit the Id-anti-Id interaction (Table 1).

One prevailing concept in immunology developed from the idiotypic network theory (15) is that the immune response to a given antigen can be regulated by a series of Id-anti-Id interactions. The expression of a given Id is under the control of an anti-Id and the anti-Id can also be regulated by an anti-anti-Id. This complex set of interactions operates via a feedback mechanism to either enhance or suppress the immune response (i.e. formation of antibodies with a particular idiotype). Our previous investigations on the immune response to PRRSV indicate the existence of the Id network (17,41). However, the role(s) of the Id-anti-Id networks in controlling the immune response to PRRSV is (are) not completely known at this time.

In an anti-idiotypic cascade, the reference starting point is an antibody termed Ab1, the combining site of which is reactive with a particular antigen and characterized by a set of idiotopes that collectively comprise its idiotype (Id). Polyclonal or monoclonal anti-Ids constitute an Ab2 population, which is used to induce an anti-anti-idiotypic or Ab3 response. If one takes into account both the specificity and idiotypic characteristics of Ab1 molecules, four discrete types of Ab3 molecules may be anticipated: Id- Ag-, Id+ Ag-, Id- Ag+, Id+ Ag+ (8). The latter includes antibodies that resemble Ab1 by reacting with the same antigen: they are designated Ab1' and the Ab2 inducing them is termed the internal image of the antigen. To further study of Id network in PRRSV infection, monoclonal anti-Id (M8G) was used to induce an anti-anti-idiotypic antibodies or Ab3 response in murine model. The binding of anti-anti-Id induced to M8G was inhibited with PRRSV antigen (Fig.5). Furthermore, the anti-anti-Id antibodies (Ab3) induced with M8G, competed with Ab1 binding to PRRSV antigen (Fig. 6) indicating that anti-anti-Id mimics the structure of the epitope in GP5 which

was recognized with Ab1. Additionally, the facts that the binding of MAb1 to MAb2 was inhibited with Ab3 (Table 3), show that monoclonal anti-Id produced the anti-anti-Id that possessed Ab1-like (Ab1') properties with specificity for the PRRSV antigen in murine model.

The relationship between Ab1 and Ab1' antibodies has been explored in a number of different antigenic systems, and with either monoclonal or polyclonal anti-Id reagents (3). One result obtained consistently is that among Ab3 antibodies are some which do indeed closely resemble the Ab1 in that they possess the same idiotopes, bind to the same antigen and derive from similar variable (V) genes. However, whilst the resemblance between Ab1' and Ab1 can be very close, many permutations of results have been seen. For example, binding of Ab3 to the antigen may not occur even although the same V-gene segments are selected, e.g. where the selection by anti-Id is directed towards the V segment rather than D and J idiotopes; or the affinity of Ab1' antibodies for antigen may be lower than that of the Ab1, corresponding to differences in the complementarity determining region (CDR), particularly H3 sequences; or the Ab3 may bind a similar antigen, yet diverge considerably from the Ab1 in V genes and CDRs. Thus, depending perhaps on the nature of the Ab2 reagent, an Ab3 population can be more or less heterogeneous and include molecules with a range of fidelities to the Ab1.

According to Goldbaum et al. (12), there are two major possibilities for the induction of anti-anti-Id (Ab3) that resemble Ab1. One is that a suitably selected anti-Id will mimic the antigen so closely as to produce the equivalent of an antibody response to the original antigen. A second possibility is that the anti-Id is specific for the Id and will thus stimulate lymphocytes

displaying anti-anti-Id immunoglobulins that should be identical or essentially identical to the Ab1. In our study, results seem to favor the second hypothesis, since anti-GP5 Ab3 showed greater binding equilibrium constants for the anti-Id than for the nominal antigen. The production and characterization of Ab3 confirms our findings that immunization with anti-GP 25C to modulate the idiotypic cascade by eliciting Ab2β antibodies that, in turn, have the capacity to stimulate specific B cell clones to secrete Ab3 antibodies. Investigations using a murine model system are in progress to determine whether the anti-Id M8G can modulate the immune response to PRRSV.

Acknowledgments

We would like to thank Dr. Kenneth Platt for providing us with the monoclonal 25C and Dana Skibbe for her technical assistance of performing IFA.

References

1. Bautista, E.M., J.J.M. Meulenberg, C.S. Choi, et al. 1996. Structural polypeptides of the American (VR-2332) strain of porcine reproductive and respiratory syndrome virus. Arch. Virol. 141:1357-1365.
2. Benfield, D.A., E.A. Nelson, J.E. Collins, et al. 1992. Characterization of swine infertility and respiratory syndrome (SIRS) virus (isolate ATCC VR-2332). J. Vet. Diagn. Investig. 4:127-133.
3. Bhattacharya-Chatterjee, M., S.K. Chatterjee, and K.A. Foon. 2000. Anti-idiotype vaccine against cancer. Immunol. Lett. 74:51-8.
4. Bona, C.A., C. Victor-Kobrin, A.J. Manheimer, et al. 1984. Regulatory arms of the immune network. Immunol. Rev. 79:25-44.
5. Bøtner, A., B. Strandbygaard, K.J. Sørensen, et al. 1997. Appearance of acute PRRS-like symptoms in sow herds after vaccination with a modified live PRRS vaccine. Vet. Rec. 141:497-499.
6. Cavanagh, D. 1997. *Nidovirales*: a new order comprising *Coronaviridae* and *Arteriviridae*. Arch.Virol. 142:629-633.
7. Christopher-Hennings, J., E.A. Nelson, R.J. Hines, et al. 1995. Persistence of porcine reproductive and respiratory syndrome virus in serum and semen of adult boars. J. Vet. Diagn. Investig. 7:456-464.
8. Corbet, S., M. Hirn, C. Roth, et al. 1988. Allogeneic manipulation of the GAT idiotypic cascade. Immunization of C57BL/6 mice by BALB/c anti-idiotypes stimulates similar strain-specific V genes as the original antigen. J. Immunol. 141:779-784.

9. Dea, S., C.A. Gagnon, H. Mardassi, et al. 2000. Current knowledge on the structural proteins of porcine reproductive and respiratory syndrome (PRRS) virus: comparison of the North American and European isolates. Arch.Virol. 145:659-688.

10. Drew, T.W., J.J.M. Meulenberg, J.J. Sands, et al. 1995. Production, characterization and reactivity of monoclonal antibodies to porcine reproductive and respiratory syndrome virus. J. Gen. Virol. 76:1361-1369.

11. Frey, M.L., K.A. Eernisse, J.G. Landgraf, et al. 1992. Diagnostic testing for SIRS virus at the National Veterinary Services Laboratories (NVSL). Am. Assoc. Swine Pract. Newslett. 4:31.

12. Goldbaum, F.A., C.A. Velikovsky, W. Dall'Acqua, et al.1997. Characterization of anti-anti-idiotypic antibodies that bind antigen and an anti-idiotype. Proc. Natl. Acad. Sci. USA 94:8697-8701.

13. Gradil, C., C. Dubuc, and M.D. Eaglesome. 1996. Porcine reproductive and respiratory syndrome virus: seminal transmission. Vet. Rec. 138:521-522.

14. Harlow, E., and D. Lane. 1988. Antibodies: a laboratory manual. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

15. Jerne, N.K. 1974. Towards a network theory of the immune system. Ann. Immunol. (Paris). 125C:373-389.

16. Jerne, N.K., J. Roland, and P.A. Cazenva. 1982. Recurrent idiotopes and internal images. EMBO. J. 1:243-247.

17. Jiang, Z., E-.M. Zhou, M. Ameri-Mahabadi, et al. 2003. Identification and characterization of auto-anti-idiotypic antibodies specific for antibodies against porcine

reproductive and respiratory syndrome virus envelope glycoprotein (GP5). Vet. Immunol. Immunopathol. 92:125-135.

18. Kohler, G., H. Hengartner, and M.J. Shulman. 1978. Immunoglobulin production by lymphocyte hybridomas. Eur. J. Immunol. 2:82-8.
19. Lin, M., and E.-M. Zhou. 1995. Internal image rabbit anti-idiotypic antibody detects sheep antibodies to the bluetongue virus core protein VP7. Immunotechnology 1:151-155.
20. Loemba, H.D., S. Mounir, H. Mardassi, et al. 1996. Kinetics of humoral immune response to the major structural proteins of the porcine reproductive and respiratory syndrome virus. Arch. Virol. 141:751-761.
21. Lowry, O.H., N.J. Rosebrough, A.L. Farr, et al. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265-275.
22. Meulenberg, J.J.M., R.J. Bende, J.M. Pol, et al. 1995. Nucleocapsid protein N of Lelystad virus: expression by recombinant baculovirus, immunological properties, and suitability for detection of serum antibodies. Clin. Diagn. Lab. Immunol. 2:652-656.
23. Meulenberg, J.J.M., A. Petersen-den Besten, E.P. de Kluyver, et al. 1997. Molecular characterization of Lelystad virus. Vet. Microbiol. 55:197-202.
24. Morrison, R.B., J.E. Collins, L. Harris, et al. 1992. Serological evidence incriminating a recently isolated virus (ATCC VR-2332) as the cause of swine infertility and respiratory syndrome (SIRS). J. Vet. Diagn. Invest. 4:186-188.
25. Murtaugh, M.P., Z. Xiao, and F. Zuckermann. 2002. Immunological Responses of Swine to Porcine Reproductive and Respiratory Syndrome Virus Infection. Viral. Immun. 15:533-547.

26. Nelson, E.A., J. Christopher-Hennings, and D. Benfield. 1994. Serum immune response to the proteins of porcine reproductive and respiratory syndrome (PRRS) virus. J. Vet. Diagn. Invest. 6:410-415.

27. Nielsen, T.L., J. Nielsen, P. Have, et al. 1997. Examination of virus shedding in semen from vaccinated and from previously infected boars after experimental challenge with porcine reproductive and respiratory syndrome virus. Vet. Microbiol. 54:101-112.

28. Nisonoff, A. 1991. Idiotypes: concepts and applications. J. Immunol.147:2429-2438.

29. Ostrowski, M., J.A. Galeota, A.M. Jar, et al. 2002. Identification of neutralizing and nonneutralizing epitopes in the porcine reproductive and respiratory syndrome virus GP5 ectodomain. J. Virol. 76:4241-4250.

30. Park, B.K., H.S. Joo, S.A. Dee, et al. 1995. Evaluation of an indirect fluorescent IgM antibody test for the detection of pigs with recent infection of porcine reproductive and respiratory syndrome virus. J. Vet. Diagn. Invest.7:544-546.

31. Pirzadeh, B., and S. Dea. 1997. Monoclonal antibodies to the ORF5 product of porcine reproductive and respiratory syndrome virus define linear neutralizing determinants. J. Gen. Virol. 78:1867-1873.

32. Snijder, E.J. and J.J.M. Meulenberg. 1998. The molecular biology of arteriviruses. J. Gen. Virol. 79:961-979.

33. Storgaard, T., M. Oleksiewicz, and A. Bøtner. 1999. Examination of the selective pressures on a live PRRS vaccine virus. Arch. Virol. 144:2389-2401.

34. van Woensel, P.A., K. Liefkens, and S. Demaret. 1998. Effect on viraemia of an American and a European serotype PPRSV vaccine after challenge with European wild-type strains of the virus. Vet. Rec. 142:510-512.

35. Weiland, E., M. Wieczorek-Krohmer, D. Kohl, et al. 1999. Monoclonal antibodies to the GP5 of porcine reproductive and respiratory syndrome virus are more effective in virus neutralization than monoclonal antibodies to the GP4. Vet. Microbiol. 66:171-186.

36. Wieczorek-Krohmer, M., F. Weiland, K.K. Conzelmann, et al. 1996. Porcine reproductive and respiratory syndrome virus (PRRSV): monoclonal antibodies detect common epitopes on two viral proteins of European and US isolates. Vet. Microbiol. 51:257-266.

37. Yang, L., M.L. Frey, K.-J. Yoon, et al. 2000. Categorization of North American porcine reproductive and respiratory syndrome viruses: epitopic profiles of the N, M, GP5 and GP3 proteins and susceptibility to neutralization. Arch. Virol. 145:1599-1619.

38. Yoon, K.J., J.J. Zimmerman, S.L. Swenson, et al. 1995. Characterization of the humoral immune response to porcine reproductive and respiratory syndrome (PRRS) virus infection. J. Vet. Diagn. Invest. 7:305-312.

39. Zhou, E.-M., T.C. Chanh, G.R. Dreesman, et al. 1987. Immune response to human immunodeficiency virus: in vivo administration of anti-idiotype induces an anti-gp 160 response specific for a synthetic peptide. J. Immunol. 139:2950-2956.

40. Zhou, E.-M., and M.K.H. Chan. 1996. Immunoblot assay as a confirmatory test of competitive ELISA for serodiagnosis of bluetongue. J. Vet. Diagn. Invest. 8:365-367.

41. Zhou, E-.M., A. Clavijo, Z. Jiang, et al. 2004. Induction of auto-anti-idiotypic antibodies specific for antibodies to matrix and envelope glycoplrotein from pigs experimentally infected with porcine reproductive and respiratory syndrome virus. Vet. Immunol. Immunopathol. 101:49-59.

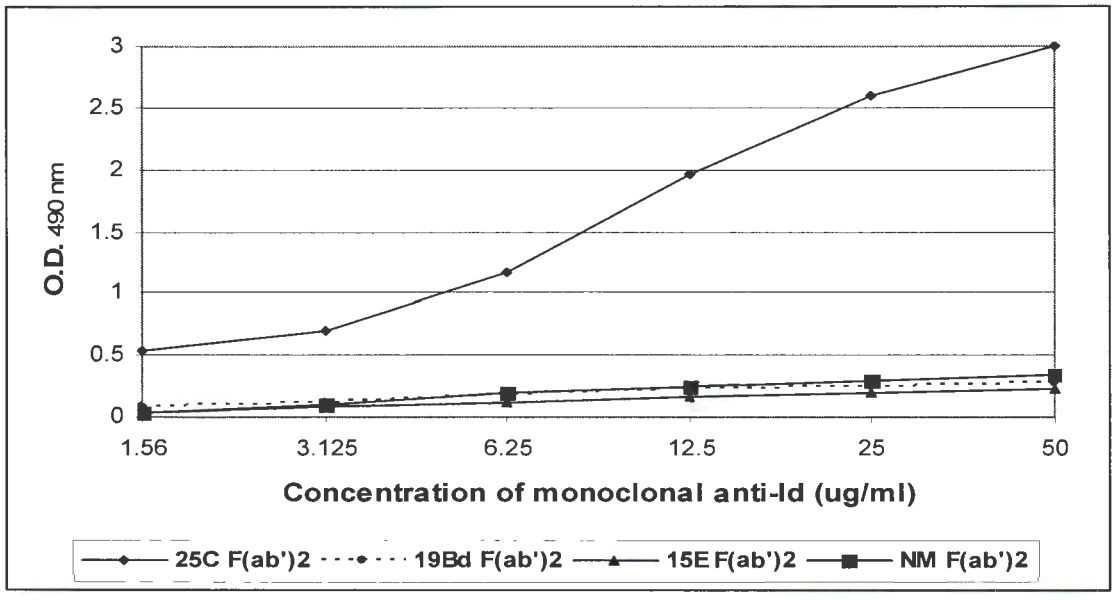

FIGURE 1. Binding curve of monoclonal anti-Id-M8G to MAb1-25C $F(ab')_2$ fragments. The solid phase was prepared by coating the wells of ELISA plates with 25C-$F(ab')_2$ at 2 μg/ml. As a control, normal mouse $F(ab')_2$ and two other $F(ab')_2$ fragments of monoclonal antibodies against matrix (19Bd) and nucleoprotein (15E) of PRRSV were similarly used. After removal of excess antibodies by washing the plate, purified monoclonal anti-Id-M8G at different concentrations was allowed to react with solid-phase $F(ab')_2$ fragments. Each point represents the mean (SEM) of duplicate values.

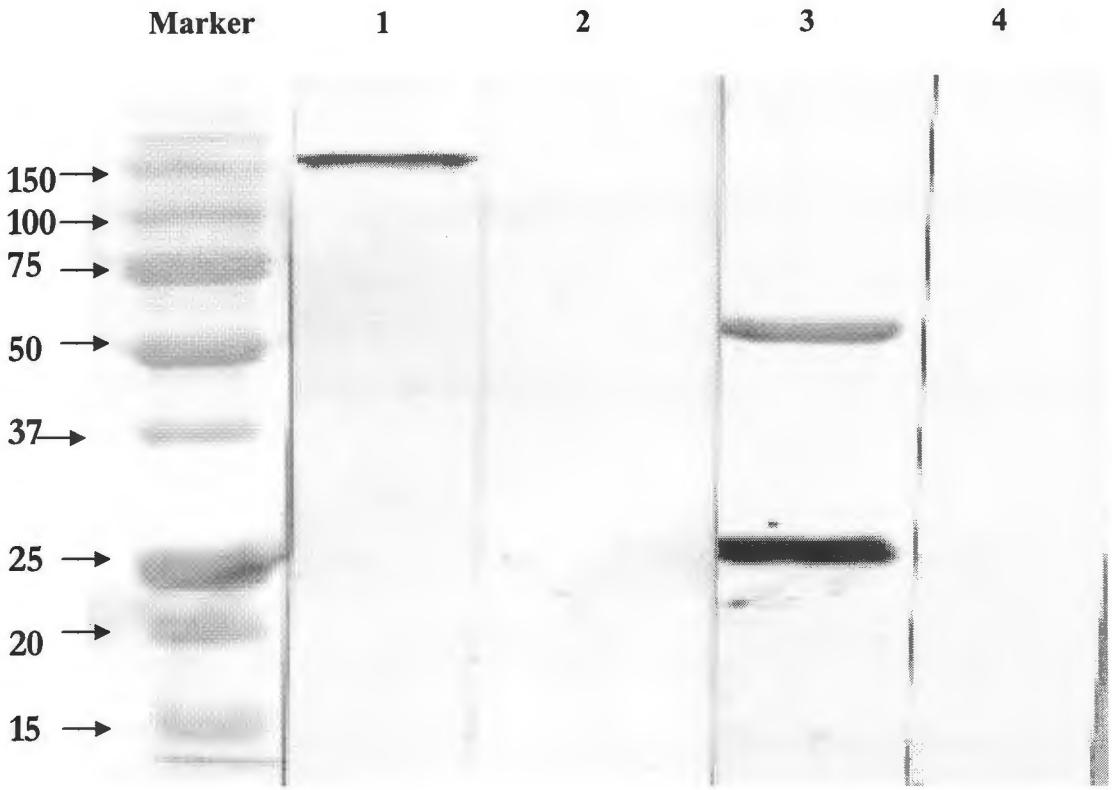

FIGURE 2. Western blot analysis of MAb2. MAb1-25C IgG (lanes 1 and 3) and normal mouse IgG (lanes 2 and 4) at 500 ng/lane were immobilized on PVDF membrane at both non-reduced (lanes 1 and 2) and reduced (lanes 3 and 4) conditions and then probed with HRP-M8G.

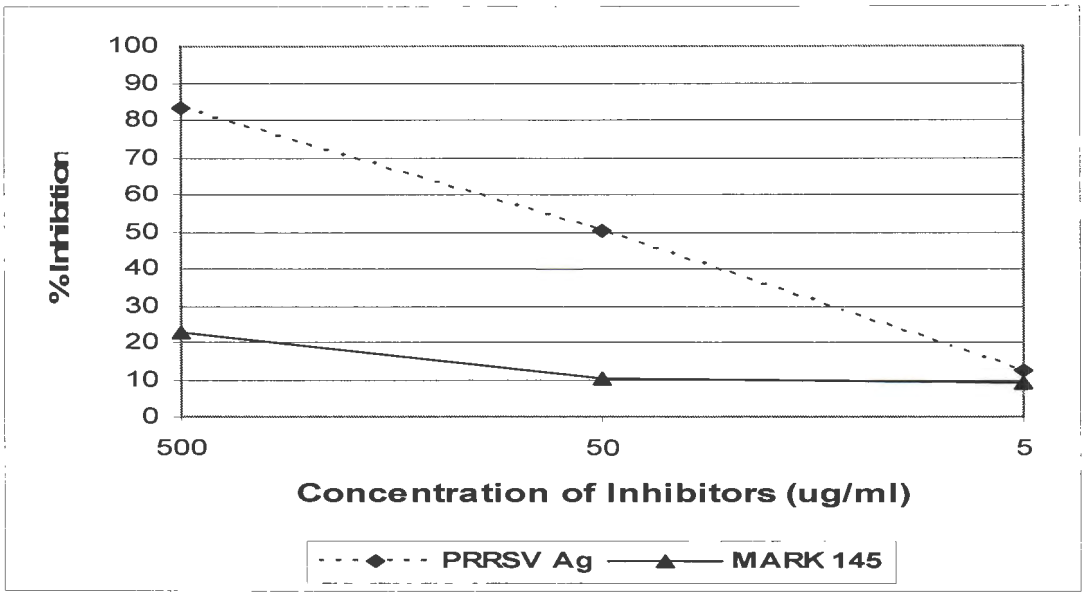

FIGURE 3. Inhibition of the binding of MAb1-25C to anti-Id-M8G-F(ab')$_2$ fragments. PRRSV antigen along with MARK 145 cell control antigen at different concentrations, were used to inhibit biotinylated 25C binding to the solid-phase M8G. Data represent the mean (SEM) of percent inhibition obtained from duplicate ELISA results.

Table 1. Inhibition of binding of MAb1 to monoclonal anti-Id [a] by various pig Ab1s

Pig no.	Serum Dilution	% Inhibition
P3	1/10	25.6 [b]
	1/100	13.5
P4	1/10	29.5
	1/100	19.6
P5	1/10	20.7
	1/100	16.9
P6	1/10	25.4
	1/100	15.5
P9	1/10	20.4
	1/100	14.4
Pre-immune	1/10	5.8
	1/100	3.3

[a] Two hundred nanograms of M8G- $F(ab')_2$ was coated to the solid phase and two different dilutions of individual swine antisera were used to inhibit the binding of biotinylated 25C to M8G.

[b] Each number represents the mean (SEM) of the percent inhibition obtained from duplicate ELISA results.

Table 2: Inhibition of binding of MAb1-25C to PRRSV by anti-Id 8G using IFA

Inhibitor	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml
MAb2-M8G	++++ [a]	+++	++	+
NM IgG	-	-	-	-

MAb against GP5 (25C) at 4 µg/ml premixed with anti-Id (M8G), and normal mouse IgG (NM IgG) at different concentrations were added to the infected cells with PRRSV cultured on slides. Binding of 25C to PRRSV was visualized using FITC-goat anti mouse IgG.

[a] "++++", "+++", "++", "+", and "-", indicated 100, 75, 50, 25 and 0 % inhibition, respectively.

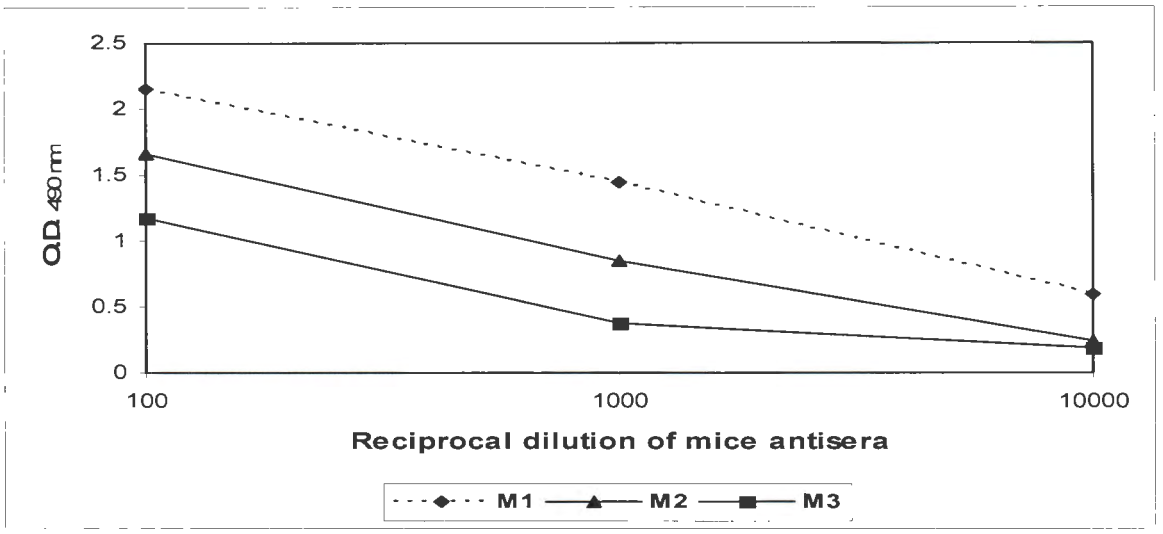

FIGURE 4. Binding curves of mice immune sera to monoclonal anti-Id M8G. Immune sera from mice immunized with anti-Id M8G-Fab were diluted in PBS-T and tested for binding to the solid phase M8G-F(ab')$_2$ by an indirect ELISA. Each point represents the mean value (SEM) of the OD490 obtained from duplicate ELISA results.

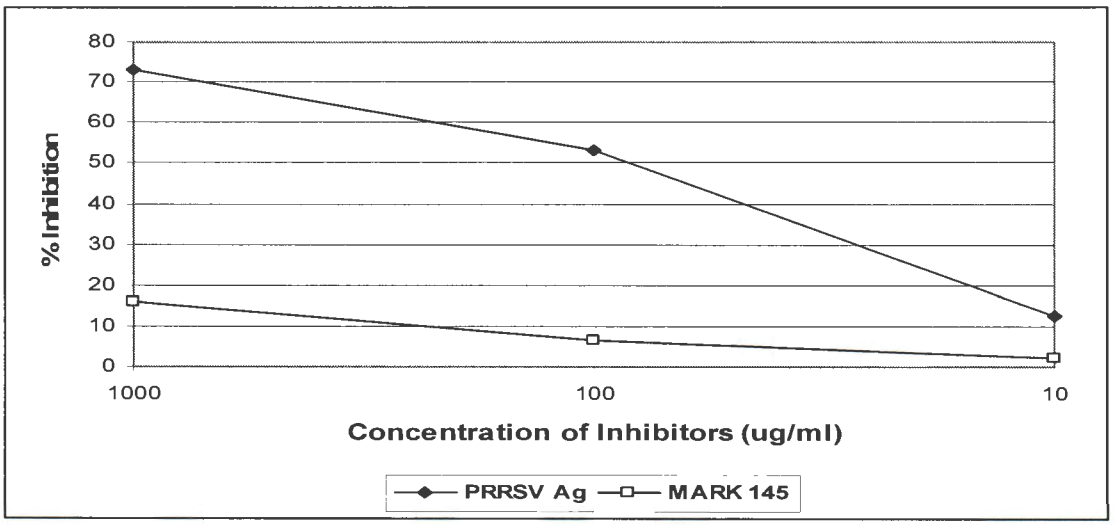

FIGURE 5. Inhibition of the binding of mouse immune serum (M1) to anti-Id M8G-F(ab')$_2$ fragments. PRRSV antigen along with MARK 145 cell control antigen at different concentrations, were used to inhibit binding of muse serum immunized with M8G to the solid-phase M8G. Each point represents the mean value (SEM) of the OD490 obtained from duplicate ELISA results.

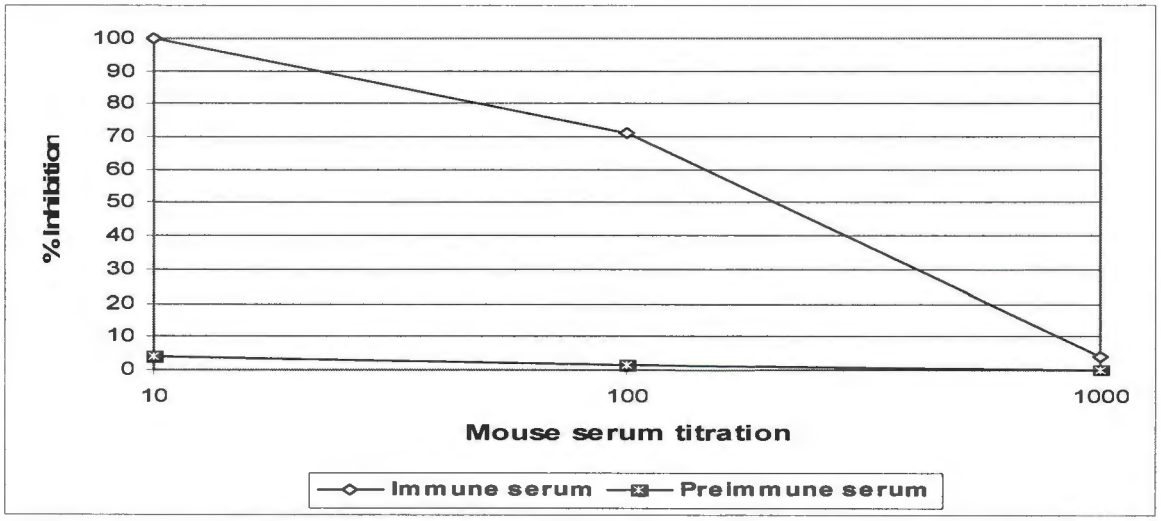

FIGURE 6. Inhibition of the binding of MAb1-25C to PRRSV antigen. Immune serum from mouse immunized with monoclonal anti-Id M8G (M1) at different dilutions, were used to inhibit MAb1-25C binding to the solid-phase PRRSV antigen. Each point represents the mean value (SEM) of the OD490 obtained from duplicate ELISA results.

Table 3. Inhibition of Id-anti-Id [a] reaction by mouse immune serum (Ab3)

Pig no.	Serum Dilution	% Inhibition
M1	1/10	86 [b]
	1/100	25
M2	1/10	39
	1/100	14.3
M3	1/10	30
	1/100	10.5
Pre-immune	1/10	3.3
	1/100	1.8

[a] Two hundred nanograms of M8G-F(ab')$_2$ was coated to the solid phase and two different dilutions of mice immune sera designated as M1, M2, or M3, as well as pre-immune serum were used to inhibit the binding of at biotinylated 25C (at 1/10000 dilution) to M8G-F(ab')$_2$ (at 2 μg/ml).

[b] Data represents the mean value (SEM) of the OD490 obtained from duplicate ELISA results.

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CHAPTER 3. Swine Auto-anti-Idiotypic Antibody Specific for Antibodies Against GP5 Antigen of Porcine Reproductive and Respiratory Syndrome Virus Elicits Specific Immune Response in Mice

A paper to be submitted to the Journal of Immunology

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Abstract

We previously identified pig auto anti-idiotypic antibody (AAb2) specific for the porcine reproductive and respiratory syndrome virus (PRRSV) glycoprotein 5 (GP5), and characterized AAb2 as an internal image anti-idiotypic antibody (Ab2β). In this study, BALB/c mice were immunized with AAb2 and the immune sera (Ab3) were tested for the presence of anti-GP5 antibodies and the expression of the idiotype of Ab1 specific for GP5 of PRRSV. Mice immunized with AAb2 were able to produce Ab1-like antibody (Id^+Ag^+) responses, i.e., they recognized the same or a similar epitope as Ab1 and possessed the Ab1 Id, without subsequent exposure to the original antigen. This was demonstrated by following results (i) inhibition of the binding of mouse immune serum to AAb2 by PRRSV antigen, (ii)

inhibition of binding of mice immune serum to PRRSV antigen by pig antisera, (iii) inhibition of AAb2 binding to MAb1-25C by Ab3, and (iv) inhibition of binding of mouse immune serum to AAb2 by pig antisera. We conclude that pig auto-anti-idiotypic antibody serologically mimics an Ab1-defined GP5 epitope sufficiently to function as a surrogate antigen for inducing anti-PRRS virus GP5 responses.

Introduction

Porcine reproductive and respiratory syndrome (PRRS), caused by the PRRS virus (PRRSV), is a disease of great economic importance for the swine industry worldwide. PRRSV is a member of the family *Arteriviridae* in the order *Nidovirales* (1). PRRSV is an enveloped positive single-stranded RNA virus, which encodes an approximately 4,000-amino-acid large replicase polyprotein (open reading frame [ORF] 1a and 1b) and six structural proteins of 130 to 265 amino acids (ORFs 2 to 7) (2-3). Three of these structural proteins have been identified as envelope (25 kDa), matrix (19 kDa), and nucleocapsid (15 kDa) proteins, i.e. GP5, M, and N proteins, respectively (4-6). Three additional proteins with molecular masses of 29–30 kDa (GP2), 45–50 kDa (GP3), and 31–35 kDa (GP4) have been identified in purified Lelystad virus and are presumed to be associated with the viral envelope (5, 7-9). Among these viral proteins, GP5 is the major envelope protein and serum neutralizing antibody titers were significantly correlated with anti-GP5 titers (10-13). The humoral immune response is presumed to play an important role in resistance to reinfection and in prevention or reduction of viral spread from animal to animal, since neutralizing antibodies have the potential to clear free virus from the circulation (14).

Anti-PRRSV immunoglobulins in serum after PRRSV infection can be detected within 7 days of infection. (14-18). Neutralizing antibodies to PRRSV develop between 1 and 2 months following exposure (15-16, 18-20). Anti-PRRS virus antibodies may persist in blood for the lifetime of commercial pigs (14), and PRRSV can persist in some animals despite high levels of antiviral antibodies which this is associated with long-term, intermittent shedding or seminal excretion of the virus (21-22).

An idiotope is a single antigenic determinant that is located on the variable regions (V domain) of both antibody molecules and receptor molecules of T and B lymphocytes. Idiotopes may be either in the antigen binding site or on the framework regions of the variable domain. The sum of the idiotopes on an antibody's V domain determines its idiotype. The idiotype (Id) network theory of immune regulation offered by Jerne (23), propose that the immune response to a given antigen can be regulated by a series of Id and its serological counterpart, an anti-idiotypic antibody (anti-Id or Ab2), has been found useful for the production of diagnostic reagents and vaccines. Upon immunization with an idiotypic antibody (Ab1), the corresponding anti-Ids are elicited and can be serologically classified into four categories (Ab2α, Ab2β, Ab2γ and Ab2ε) (24-25). Ab2α recognizes an Id associated with the framework region of Ab1. Ab2β, an internal image anti-Id Ab2, recognizes an Id within the antigen-combining site and bears a structural resemblance to the original antigen. The anti-Id mimicry of the original antigen has been experimentally proved in many virus systems (26-28). Ab2γ, like Ab2β, recognizes an Id within the antigen-combining site but does not carry the internal image of the original antigen. Ab2ε is defines as the antibody that binds both Ab1 and antigen (25). A given idiotypic antibody is under the

control of an anti-Id, whereas the anti-Id can be regulated by anti-anti-Id (or Ab3). The concept of internal image stems from the possibility of raising Ab3 antibodies that would recognize the original Ag. If one takes into account both the specificity and the idiotypic characteristics of Ab1 molecules, four discrete types of Ab3 molecules may be anticipated: Id^-Ag^-, Id^+Ag^-, Id^-Ag^+ and Id^+Ag^+, the later being very similar to Ab1 and termed Ab1' (29).

PRRSV infection poses a challenge to current serodiagnostic and vaccination strategies. Because the genetic diversity of field PRRSV isolates is very high, vaccine efficacy against heterologous challenge may be limited (30). Also, live PRRSV vaccines have been observed to revert to virulence (31-32), and the safer, killed vaccines have so far proved less effective (33). A better understanding of the mechanisms that regulate immunity to this virus will aid in the development of more effective vaccines.

We have previously suggested the possible role of auto-anti-idiotypic antibodies (AAb2) in immunity to PRRSV infection and characterized pig AAb2 as an internal image anti-idiotypic antibody (Ab2β) (34-35). In the present study, we describe the characterization of an anti-anti-Id, which was produced against a pig AAb2 specific for GP5 of PRRSV and determined the structure of Ab3 (Id^+Ag^+ or Id^+Ag^-).

Materials and Methods

PRRSV antigen

A cell line, MARC-145 was infected with VR-2332 of PRRSV isolate at a multiplicity of infection of approximately 5 and the infected cell culture was then incubated and harvested

as described by Yoon et al. (16). The total protein concentration of the crude cell culture antigen preparation was determined by the method of Lowry et al. (36) using a protein assay kit (Pierce, Rockford, IL).

Antibodies

MAb1 specific for the PRRSV glycoprotein (GP5), designated 25C (IgG_1, λ), has been described elsewhere (34). Purification of AAb2 was performed as described previousely (34-35). Briefly, normal mouse IgG was covalently coupled to the activated agarose support matrix for affinity purification of swine AAb2 specific for GP5 of PRRSV using the manufacturer protocol (Bio-Rad Laboratories, Hercules, CA). Swine serums was diluted two-fold in PBS and passed repeatedly through a normal mouse IgG agarose column until anti-isotypes and anti-allotypes were completely removed and effluent was collected. The unbound serum sample was tested for binding to the solid-phase normal mouse IgG by ELISA to ensure that it was free of anti-isotypes and anti-allotypes. The effluent was then affinity purified using membrane-bound 25C-$F(ab')_2$ fragments. A PVDF membrane (Bio-Rad Laboratories, Hercules, CA) was first treated with methanol for 5 second, equilibrated in the binding buffer (Tris–HCl) and semi-dried between tissue paper. The 25C-$F(ab')_2$ at 1 mg/ml in PBS was loaded on the membrane (5 cm2) for 30 min. The 25C-$F(ab')_2$-coated membrane was then incubated in 1% BSA (w/v) in PBS-T [0.01 M PBS, pH 7.2, containing 0.01% (v/v) Tween-20] for another 30 min. After washing three times with PBS-T, the membrane was treated with the elution buffer, 0.1 M glycine, pH 5, for 5 min to remove any loose-bound $F(ab')_2$ fragments. After washing three times with PBS-T, the membrane was

then reacted with pig serum sample diluted 1:50 in PBS-T for 1 h at room temperature. After washing three times with PBS-T, the bound AAb2 were eluted with the elution buffer. This elution buffer at pH 5 was chosen because at lower pH (pH 2–3), some coated $F(ab')_2$ fragments were also eluted out along with the AAb2. The pH of AAb2 solution was immediately adjusted to pH 7 with 3 M Tris-base solution. After the elution with the elution buffer, the bound AAb2 was completely eluted out, which was evidenced by the lack of reaction of the membrane with HRP-goat anti-swine IgG (data not shown). IgG concentration was estimated at 280 nm using a spectrophotometer (SmartSpec 3000, Bio-Rad Laboratories, Hercules CA) with the extinction coefficient of 1.35. The purity of antibodies was confirmed by SDS-PAGE.

Enzymatic digestion of antibodies

To prepare $F(ab')_2$, the purified 25C-IgG and AAb2-IgG were digested using pepsin (Sigma, St. Louis, MO) as described previously (34). A papain digestion was performed to prepare AAb2-Fab based on method described elsewhere (37). The purity of $F(ab')_2$ and Fab were confirmed using SDS-PAGE and ELISA.

Pig sera

Pig antisera samples were collected from a group of pigs experimentally infected with PRRSV. The presence of anti-PRRSV antibodies was confirmed by IFA and HerdCheck ELISA as described in our previous studies (34-35).

Production of anti-anti-idiotypic antibody (Ab3) specific for GP5 of PRRSV

For induction of Ab3, AAb2 was used in the form of Fab coupled to KLH (Fab-KLH). Three, six week-old female BALB/c mice were immunized intraperitoneally (IP) on day 0, with 50 μg of AAb_2-Fab-KLH (50 μg in 100 μl of PBS) emulsified 1:1 in Freund's complete adjuvant (Total vol. = 0.2 ml). Two booster injections were given on days 14 and 28 each with 50 μg of the same anti-idiotype preparation in Freund's incomplete adjuvant. Two weeks after the final injection, mice were bled and the serum samples tested for the presence of anti-anti idiotypic antibody (Ab3) in serum by an ELISA with AAb2-Fab on solid-phase. The antibody end point titre (EPT) was expressed as the highest serum dilution that gave the absorbance at 490 nm 3-fold higher than that for pre-immune serum.

Detection of anti-anti-Id antibody (Ab3)

The presence of anti-anti-Id antibodies (Ab3) in the sera from mice immunized with AAb2 was tested by an indirect ELISA. The solid phase was prepared by coating the wells of ELISA plates (Nalge Nunc International, Roskilde, Denmark) with AAb2-$F(ab')_2$ or normal pig $F(ab')_2$ fragments at 2 μg/ml (100 μl/well) in PBS (10 mM, pH 7.2) at 4 °C, overnight. After washing the plate with PBS containing 0.05 % of Tween-20 (PBS-T, v/v), individual mouse serum were diluted in 5% normal pig serum (NPS) in PBS-T and added to the wells (100 μl/well) in duplicates and incubated for 45 min at room temperature. The wells were washed again three times with PBS-T and the presence of Ab3 that bound to the solid-phase AAb2-$F(ab')_2$ was detected by the addition of HRP-goat anti-mouse IgG (Jackson ImmunoResearch, West Grove, PA) diluted 2000-fold in PBS-T (100 μl/well) for 45 min at

room temperature. After final washing with PBS-T, the substrate *o*-phenylene diamine (OPD, 100 µl/well) was added to all wells to develop the colorimetric reaction at the room temperature for 15 min. The reaction was then stopped by adding 3 M H_2SO_4 in each well (100 µl/well) and read at OD 490 nm on an automatic ELISA plate reader (Universal Microplate Reader, EL800, Bio-Tek Instrument, Inc., Winooski, VT).

Competitive ELISAs

To determine whether PRRSV antigen could inhibit AAb2 binding to Ab3, a C-ELISA was performed by coating of AAb2-F(ab')$_2$ (0.2 µg/well) onto the ELISA plate. The mouse serum (M1), at the final concentration of 1:1000) was used in the liquid-phase with or without mixing with individual inhibitors including PRRSV antigen and MARK 145 cell line (as a control) at 100 and 10 µg/ml. After washing the plates 3 times with PBS-T, HRP-goat anti-mouse IgG diluted 2000-fold in PBS-T (100 µl/well) for 45 min at room temperature was added. After final washing with PBS-T for three times, substrate solution, OPD (100 µl/well) was added to all wells to develop the colorimetric reaction at the room temperature for 15 min. The reaction was stopped by adding 3 M H_2SO_4 in each well (100 µl/well) and the OD was read at 490 nm on an automatic ELISA plate reader. The percent inhibition was calculated by the following formula:

% INHIBITION = [1 - (OD_{490} with competitor - background OD_{490}) / (OD_{490} without competitor - background OD_{490})] × 100.

To further confirm the specificity of Ab3 for PRRSV, the ability of pig antisera (Ab1) to inhibit Ab3 from binding to the solid-phase PRRSV antigen was tested by competitive ELISA. For this purpose 96-well microtitre plates were coated with PRRSV Ag (100 µl/well), 2 µg/ml in PBS (0.01M, pH 7.2) and incubated over night at 4°C. The mouse serum immunized with AAb2 (1/10000 dilution) was used in the liquid-phase with or without mixing with individual pig antisera along with pre-immune serum at 1/10 and 1/100 dilution. 100 µ1 of the mixture were added to the wells and kept for 1 h at room temperature followed by washing three times with PBS-T. The conjugate used in the C-ELISA was HRP-goat anti-mouse IgG at 1:2000 dilution in PBS-T (100 µl/well). The addition of the substrate and quantitation of the calorimetric reaction was performed as described above. The percent of inhibition was calculated by the same formula.

Furthermore, a C-ELISA was performed to determine whether Ab3 could inhibit AAb2 binding to MAb1. For this purpose, the solid phase was prepared by coating the wells of plate with 25C-F(ab')$_2$ (2 µg/ml in PBS). The AAb2 (100 µg/ml dilution) was used in the liquid-phase with or without mixing with individual mice sera immunized with AAb2 (Ab3), at 1/100. 100 µl of the mixture were added to the wells and kept for 1 h at room temperature followed by washing three times with PBS-T. The conjugate used in the C-ELISA was HRP-goat anti-swine IgG at 1:2000 dilution in PBS-T (100 µl/well). The rest of assay was performed as the same described previously and the degree of inhibition of Id-anti-Id interaction was calculated by the formula described above.

To determine whether Ab3 express an Id of Ab1, the ability of pig antisera (Ab1) to inhibit mouse immune serum (Ab3) from binding to the solid-phase AAb2 were evaluated by an inhibition ELISA. Similarly as described above, AAb2-F(ab')$_2$ (2 μg/ml in PBS) was absorbed to wells of an ELISA plate. After washing the plate 3 times with PBS-T, serum from the mouse immunized with AAb2 (at final dilution of 1:1000 in PBS-T) was mixed with various pig sera (1:10 and 1:100 dilutions in PBS-T) and 100 μl of the mixture was added to the wells and incubated for 45 min at room temperature. The wells were washed three times with PBS-T and the inhibition of Ab3 to the AAb2 was detected by the addition of HRP-goat anti-mouse IgG diluted 2000-fold in PBS-T (100 μl/well). The plates were incubated for another 45 min at room temperature. The percent inhibition was calculated by the formula described above.

Results

Induction of anti-anti-Id antibody

Immunization with swine AAb2 in a xenogenic system was done and the induced anti-anti-Id antisera (Ab3) were analyzed with an indirect ELISA. Murine sera were diluted in PBS-T containing 5% NPS and tested for binding to the solid-phase AAb2-F(ab')$_2$ or normal pig IgG. Fig.1 shows the binding activities of three mouse serum immunized with anti-Id antibody (AAb2). Under assay conditions employed, the absorbance values at 490 nm for binding of M1, M2 and M3 immune sera at 1/10000 dilution to the solid-phase AAb2 were 2.42, 2.02 and 2.3, respectively, which were much greater than those for binding of the same

antisera to normal pig $F(ab')_2$ (Fig.1). Pre-immune sera from all immunized mice showed negligible immunoreaction with AAb2 or normal pig IgG (data not shown).

Ab3 antibodies recognize the Ab1-specific epitope of GP5

To determine whether Ab3 antibodies possessed Ab1-like properties with specificity for the PRRSV antigen, two competitive ELISA were performed. First a C-ELISA was used to determine whether PRRSV antigen could inhibit Ab3 binding to AAb2. As shown in Fig. 2, PRRSV antigen compare to MARK 145 cell line, significantly ($P<0.05$) inhibited (73 and 53 % at 1000 and 100 μg/ml, respectively) Ab3 binding to AAb2.

To further confirm the specificity of Ab3 for PRRSV antigen, the immune sera were tested additionally by the second immunoassay. A C-ELISA was employed for the ability of pig antisera to inhibit immune serum from binding to the solid-phase PRRSV antigen. As shown in Table 1, the pig antisera significantly ($P<0.05$) inhibited the Ab3-PRRSV antigen interaction. The highest and lowest percentages of inhibition were 84 and 31.4 (at a dilution of 1:10), respectively. Pig negative serum did not show any inhibitory effects. These results demonstrated that AAb2 did induce the production of anti-GP5 antibodies.

Ab3 antibodies possess the Id of Ab1

To determine whether Ab3s express an Id of Ab1, the immune sera were evaluated for their ability to inhibit AAb2 from binding to the solid-phase 25C by a C-ELISA. Figure 3 shows the percent of inhibition of Id-anti-Id reaction by mice immune sera (M1, M2, and M3) were

66, 74, and 68 %, respectively. Negligible inhibitory effects were found with the normal mouse serum (8.5 %).

Common anti-Id

To examine whether a common idiotope was present at a significant level in the Ab3 anti GP5 antibodies, the ability of pig antisera (Ab1) to inhibit mouse immune serum (Ab3) from binding to the solid-phase AAb2 by an inhibition ELISA. Table 2 shows that pig antisera at 1:10 dilution, significantly ($P<0.05$) inhibit binding of Ab3 to AAb2, while pre-immune pig serum did not affect binding of Ab3 antibodies to AAb2.

Discussion

We have previously identified pig auto anti-idiotypic antibody (AAb2) specific for the porcine reproductive and respiratory syndrome virus (PRRSV) glycoprotein GP5, and showed that AAb2 possessed the properties of an internal image anti-Id (Ab2β) (34-35). Based on Jerne's idiotype network theory (23) and confirmed by Bona and Kohler (38) anti-idiotypic antibodies can be classified into four categories: Ab2α, Ab2β, Ab2γ and Ab2ε. These individual anti-idiotypic antibodies have been produced against various antigens and used to modulate the specific immune response against the original antigens (24, 38). In particular, an internal image of anti-idiotype (Ab2β) has been studied to modulate the specific antibody responses against bluetongue virus VP2 (39) and VP7 (40), HBV (41), and HIV (28). In this study, BALB/c mice were immunized with AAb2 and the immune sera

(Ab3) were tested for the presence of anti-GP5 antibodies and the expression of the Id of Ab1 specific for GP5 of PRRSV.

One prevailing concept in immunology developed from the idiotypic network theory (23) is that the immune response to a given antigen can be regulated by a series of Id-anti-Id interactions. The expression of a given Id is under the control of an anti-Id and the anti-Id can also be regulated by an anti-anti-Id. This complex set of interactions operates via a feedback mechanism to either enhance or suppress the immune response (i.e. formation of antibodies with a particular idiotype). Our previous investigations on the immune response to PRRSV indicate the existence of the Id network (34-35). However, the role(s) of the Id-anti-Id networks in controlling the immune response to PRRSV is (are) not completely known at this time.

The results of the present study demonstrated that immunization of mice with AAb2 led to the generation of an anti-GP5 antibody response with epitope specificity similar to that of Ab1, as the interaction between anti-anti-Id and AAb2 was inhibited by PRRSV antigen (Fig.2), and the interaction between Ab3 and PRRSV antigen was inhibited by pig antisera to PRRSV antigen (Table 1). The finding that the binding of AAb2 to MAb1 (25C) was inhibited with Ab3 (Fig. 3), and pig antisera (Ab1) inhibited interaction between Ab3 and anti-Id (Table 2), show that auto-anti-Id produced the anti-anti-Id which recognized the idiotypes that were shared by mouse and pig anti-PRRSV antibodies. Because the antibodies to GP5 in the swine sera are of polyclonal origin and, therefore, are capable of recognizing any of the determinants of PRRSV e.g. M and N proteins and or other glycoproteins (GP2,

Gp3, GP4), it is evident that only that fraction of the entire repertoire of antibodies which is (i) directed to GP5 of PRRSV and (ii) possesses the Id which is the same or similar to that of 25C, has the capacity to inhibit the Id-anti-Id interaction. Therefore, it was not surprising to find that the swine sera were not able to completely inhibit the Ab3-AAb2 interaction.

The relationship between Ab1 and Ab1' antibodies has been explored in a number of different antigenic systems, and with either monoclonal or polyclonal anti-Id reagents (42). One result obtained consistently is that among Ab3 antibodies are some antibodies that do indeed closely resemble the Ab1 in that they possess the same idiotopes, bind to the same antigen and derive from similar variable (V) genes. However, whilst the resemblance between Ab1' and Ab1 can be very close, many permutations of results have been seen. For example, binding of Ab3 to the antigen may not occur even although the same V-gene segments are selected, e.g. where the selection by anti-Id is directed towards the V segment rather than D and J idiotopes; or the affinity of Ab1' antibodies for antigen may be lower than that of the Ab1, corresponding to differences in the complementarity determining region (CDR), particularly H3 sequences; or the Ab3 may bind a similar antigen, yet diverge considerably from the Ab1 in V genes and CDRs. Thus, depending perhaps on the nature of the Ab2 reagent, an Ab3 population can be more or less heterogeneous and include molecules with a range of fidelities to the Ab1.

According to Goldbaum et al. (43), there are two major possibilities for the induction of anti-anti-Id (Ab3) that resemble Ab1. One is that a suitably selected anti-Id will mimic the antigen so closely as to produce the equivalent of an antibody response to the original antigen. A

second possibility is that the anti-Id is specific for the Id and will thus stimulate lymphocytes displaying anti-anti-Id immunoglobulins that should be identical or essentially identical to the Ab1. By immunochemical criteria it is difficult to discriminate between Ab2β and Ab2γ. To sort out both types of Ab2 a functional demonstration should be observed. Since Ab2β express an idiotype that mimics the antigen, it might have the capacity to elicit antibodies specific for the antigen in the same or different species. The production and characterization of Ab3 confirms our findings that immunization with anti-GP 25C to modulate the idiotypic cascade by eliciting Ab2β antibodies that, in turn, have the capacity to stimulate specific B cell clones to secrete Ab3 antibodies. The mouse serum containing Ab3 recognized specifically PRRSV antigen in EIA assays, showing a typical saturation pattern. Moreover, Ab3 recognized a common Id on the pig antisera against GP5. Investigations using a murine model system are in progress to determine whether the swine AAb2 can modulate the immune response to PRRSV.

Acknowledgments

We would like to thank Dr. Kenneth Platt for providing monoclonal 25C.

References

1. Cavanagh, D. 1997. *Nidovirales*: a new order comprising *Coronaviridae* and *Arteriviridae*. *Arch. Virol. 142:629.*
2. Dea, S., C.A. Gagnon, H. Mardassi, B. Pirzadeh, and D. Rogan. 2000. Current knowledge on the structural proteins of porcine reproductive and respiratory syndrome (PRRS) virus: comparison of the North American and European isolates. *Arch. Virol. 145:659.*
3. Snijder, E.J., and J.J.M. Meulenberg. 1998. The molecular biology of arteriviruses. *J. Gen. Virol. 79:961.*
4. Benfield, D.A., E.A. Nelson, J.E. Collins, L. Harris, S.M. Goyal, D. Robison, W.T. Christianson, R.B. Morrison, D. Gorcyca, and D.W. Chladek. 1992. Characterization of swine infertility and respiratory syndrome (SIRS) virus (isolate ATCC VR-2332). *J. Vet. Diagn. Investig. 4:127.*
5. Meulenberg, J.J.M., R.J. Bende, J.M. Pol, G. Wensvoort, and R.J.M. Moormann. 1995. Nucleocapsid protein N of Lelystad virus: expression by recombinant baculovirus, immunological properties, and suitability for detection of serum antibodies. *Clin. Diagn. Lab. Immunol. 2:652.*
6. Bautista, E.M., J.J.M. Meulenberg, C.S. Choi, and T.W. Molitor. 1996. Structural polypeptides of the American (VR-2332) strain of porcine reproductive and respiratory syndrome virus. *Arch. Virol. 141:1357.*
7. Drew, T.W., J.J.M. Meulenberg, J.J. Sands, and D.J. Paton. 1995. Production, characterization and reactivity of monoclonal antibodies to porcine reproductive and respiratory syndrome virus. *J. Gen. Virol. 76:1361.*

8. Meulenberg, J.J.M., A. Petersen-den Besten, E.P. de Kluyver, A.P. van Nieuwstadt, G. Wensvoort, and R.J.M. Moormann. 1997. Molecular characterization of Lelystad virus. *Vet. Microbiol. 55:197.*

9. Wieczorek-Krohmer, M., F. Weiland, K.K. Conzelmann, D. Kohl, N.Visser, P. Van Woensel, H.J. Thiel, and E.Weiland. 1996. Porcine reproductive and respiratory syndrome virus (PRRSV): monoclonal antibodies detect common epitopes on two viral proteins of European and US isolates. *Vet. Microbiol. 51:257.*

10. Ostrowski, M., J.A. Galeota, A.M. Jar, K.B. Platt, F.A. Osorio, and O.J. Lopez. 2002. Identification of neutralizing and nonneutralizing epitopes in the porcine reproductive and respiratory syndrome virus GP5 ectodomain. *J. Virol. 76:4241.*

11. Yang, L., M.L. Frey, K.-J. Yoon, J.J. Zimmerman, and K.B. Platt. 2000. Categorization of North American porcine reproductive and respiratory syndrome viruses: epitopic profiles of the N, M, GP5 and GP3 proteins and susceptibility to neutralization. *Arch. Virol. 145:1599.*

12. Weiland, E., M. Wieczorek-Krohmer, D. Kohl, K.K. Conzelmann, F. Weiland. 1999. Monoclonal antibodies to the GP5 of porcine reproductive and respiratory syndrome virus are more effective in virus neutralization than monoclonal antibodies to the GP4. *Vet. Microbiol. 66:171.*

13. Pirzadeh, B., and S. Dea. 1997. Monoclonal antibodies to the ORF5 product of porcine reproductive and respiratory syndrome virus define linear neutralizing determinants. *J. Gen. Virol. 78:1867.*

14. Murtaugh, M.P., Z. Xiao, and F. Zuckermann. 2002. Immunological Responses of Swine to Porcine Reproductive and Respiratory Syndrome Virus Infection. *Viral. Immun. 15:533.*

15. Loemba, H.D., S. Mounir, H. Mardassi, D. Archambault, and S. Dea. 1996. Kinetics of humoral immune response to the major structural proteins of the porcine reproductive and respiratory syndrome virus. *Arch. Virol. 141:751.*

16. Yoon, K.J., J.J. Zimmerman, S.L. Swenson, M.J. McGinley, K.A. Eernisse, A. Brevik, L.L. Rhinehart, M.L. Frey, H.T. Hill, and K.B. Platt. 1995. Characterization of the humoral immune response to porcine reproductive and respiratory syndrome (PRRS) virus infection. *J. Vet. Diagn. Invest. 7:305.*

17. Park, B.K., H.S. Joo, S.A. Dee, and C. Pijoan. 1995. Evaluation of an indirect fluorescent IgM antibody test for the detection of pigs with recent infection of porcine reproductive and respiratory syndrome virus. *J. Vet. Diagn. Invest.7:544.*

18. Nelson, E.A., J. Christopher-Hennings, and D. Benfield. 1994. Serum immune response to the proteins of porcine reproductive and respiratory syndrome (PRRS) virus. *J. Vet. Diagn. Invest. 6:410.*

19. Frey, M.L., K.A. Eernisse, J.G. Landgraf, J. Pearson, and D. Chladek. 1992. Diagnostic testing for SIRS virus at the National Veterinary Services Laboratories (NVSL). *Am. Assoc. Swine Pract. Newslett. 4:31.*

20. Morrison, R.B., J.E. Collins, L. Harris, W.T. Christianson, D.A. Benfield, D.W. Chladek, D.E. Gorcyca, and H.S. Joo. 1992. Serological evidence incriminating a recently isolated

virus (ATCC VR-2332) as the cause of swine infertility and respiratory syndrome (SIRS). *J. Vet. Diagn. Invest. 4:186.*

21. Christopher-Hennings, J., E.A. Nelson, R.J. Hines, J.K. Nelson, S.L. Swenson, J.J. Zimmerman, C.L. Chase, M.J. Yaeger, and D.A. Benfield. 1995. Persistence of porcine reproductive and respiratory syndrome virus in serum and semen of adult boars. *J. Vet. Diagn. Investig. 7:456.*
22. Gradil, C., C. Dubuc, and M.D. Eaglesome. 1996. Porcine reproductive and respiratory syndrome virus: seminal transmission. *Vet. Rec. 138:521.*
23. Jerne, N.K. 1974. Towards a network theory of the immune system. *Ann. Immunol. (Paris). 125C:373.*
24. Jerne, N.K., J. Roland, and P.A. Cazenva. 1982. Recurrent idiotopes and internal images. *EMBO. J. 1:243.*
25. Bona, C.A., C. Victor-Kobrin, A.J. Manheimer, B. Bellon, and L.J. Rubinstein. 1984. Regulatory arms of the immune network. *Immunol. Rev. 79:25.*
26. Lin, M., and E.-M. Zhou. 1995. Internal image rabbit anti-idiotypic antibody detects sheep antibodies to the bluetongue virus core protein VP7. *Immunotechnology 1:151.*
27. Nisonoff, A. 1991. Idiotypes: concepts and applications. *J. Immunol.147:2429.*
28. Zhou, E.-M., T.C. Chanh, G.R. Dreesman, P. Kanda, and R.C. Kennedy. 1987. Immune response to human immunodeficiency virus: in vivo administration of anti-idiotype induces an anti-gp 160 response specific for a synthetic peptide. *J. Immunol.139:2950.*
29. Corbet, S., M. Hirn, C. Roth, J. Theze, M. Fougereau, and C. Schiff. 1988. Allogeneic manipulation of the GAT idiotypic cascade. Immunization of C57BL/6 mice by BALB/c

anti-idiotypes stimulates similar strain-specific V genes as the original antigen. *J. Immunol. 141:779.*

30. van Woensel, P.A., K. Liefkens, and S. Demaret. 1998. Effect on viraemia of an American and a European serotype PPRSV vaccine after challenge with European wild-type strains of the virus. *Vet. Rec. 142:510.*
31. Storgaard, T., M. Oleksiewicz, and A. Bøtner. 1999. Examination of the selective pressures on a live PRRS vaccine virus. *Arch. Virol. 144:2389.*
32. Bøtner, A., B. Strandbygaard, K.J. Sørensen, P. Have, K.G. Madsen, E. Smedegaard Madsen, and S. Alexandersen. 1997. Appearance of acute PRRS-like symptoms in sow herds after vaccination with a modified live PRRS vaccine. *Vet. Rec. 141:497.*
33. Nielsen, T.L., J. Nielsen, P. Have, P. Baekbo, J.R. Hoff, and A. Botner. 1997. Examination of virus shedding in semen from vaccinated and from previously infected boars after experimental challenge with porcine reproductive and respiratory syndrome virus. *Vet. Microbiol. 54:101.*
34. Jiang, Z., E-.M. Zhou, M. Ameri-Mahabadi, J.J. Zimmerman, and K.B. Platt. 2003. Identification and characterization of auto-anti-idiotypic antibodies specific for antibodies against porcine reproductive and respiratory syndrome virus envelope glycoprotein (GP5). *Vet. Immunol. Immunopathol. 92:125.*
35. Zhou, E-.M., A. Clavijo, Z. Jiang, M. Ameri-Mahabadi, and J.J. Zimmerman. 2004. Induction of auto-anti-idiotypic antibodies specific for antibodies to matrix and envelope glycoplrotein from pigs experimentally infected with porcine reproductive and respiratory syndrome virus. *Vet. Immunol. Immunopathol. 101:49.*

36. Lowry, O.H., N.J. Rosebrough, A.L. Farr, and R.J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem. 193: 265.*

37. Harlow, E., and D. Lane. 1988. Antibodies: a laboratory manual. *Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.*

38. Bona, C.A., and H. Kohler. 1984. Anti-idiotypic antibodies and internal images. In: Venter, J.C., Fraser, C.M., Linstrom, J. (Eds.), Monoclonal and Anti-idiotypic Antibodies: Probes for Receptor Structure and Function. *Springer, New York, pp. 141.*

39. Grieder, F.B., and K.T. Schultz. 1990. Antiidiotypic antibody mimicry of a bluetongue virus neutralizing antigen. *J. Immunol. 7:2627.*

40. Zhou, E.-M., and M. Lin. 1997. Anti-idiotype to bluetongue virus VP7 antigen: potential diagnostic reagent and vaccine. In: Shoenfeld, Y., Kennedy, R.C., Ferrone, S. (Eds.), Idiotypes in Medicine: Autoimmunity, Infection and Cancer. *Elsevier, Amsterdam, The Netherlands, pp. 347.*

41. Kennedy, R.C., and G.R. Dreesman, 1986.Anti-Idiotypic Antibodies as Idiotope Vaccines that Induce Immunity against Infectious Agents, Intern. Rev. Immunol. 1:6778.

42. Bhattacharya-Chatterjee M., S.K. Chatterjee, and K.A. Foon. 2000. Anti-idiotype vaccine against cancer. *Immunol. Lett. 74:51.*

43. Goldbaum, F.A., C.A. Velikovsky, W. Dall'Acqua, C.A. Fossati, B.A. Fields, B.C. Braden, R.J. Poljak, and R.A. Mariuzza. 1997. Characterization of anti-anti-idiotypic antibodies that bind antigen and an anti-idiotype. *Proc. Natl. Acad. Sci. USA 94:8697.*

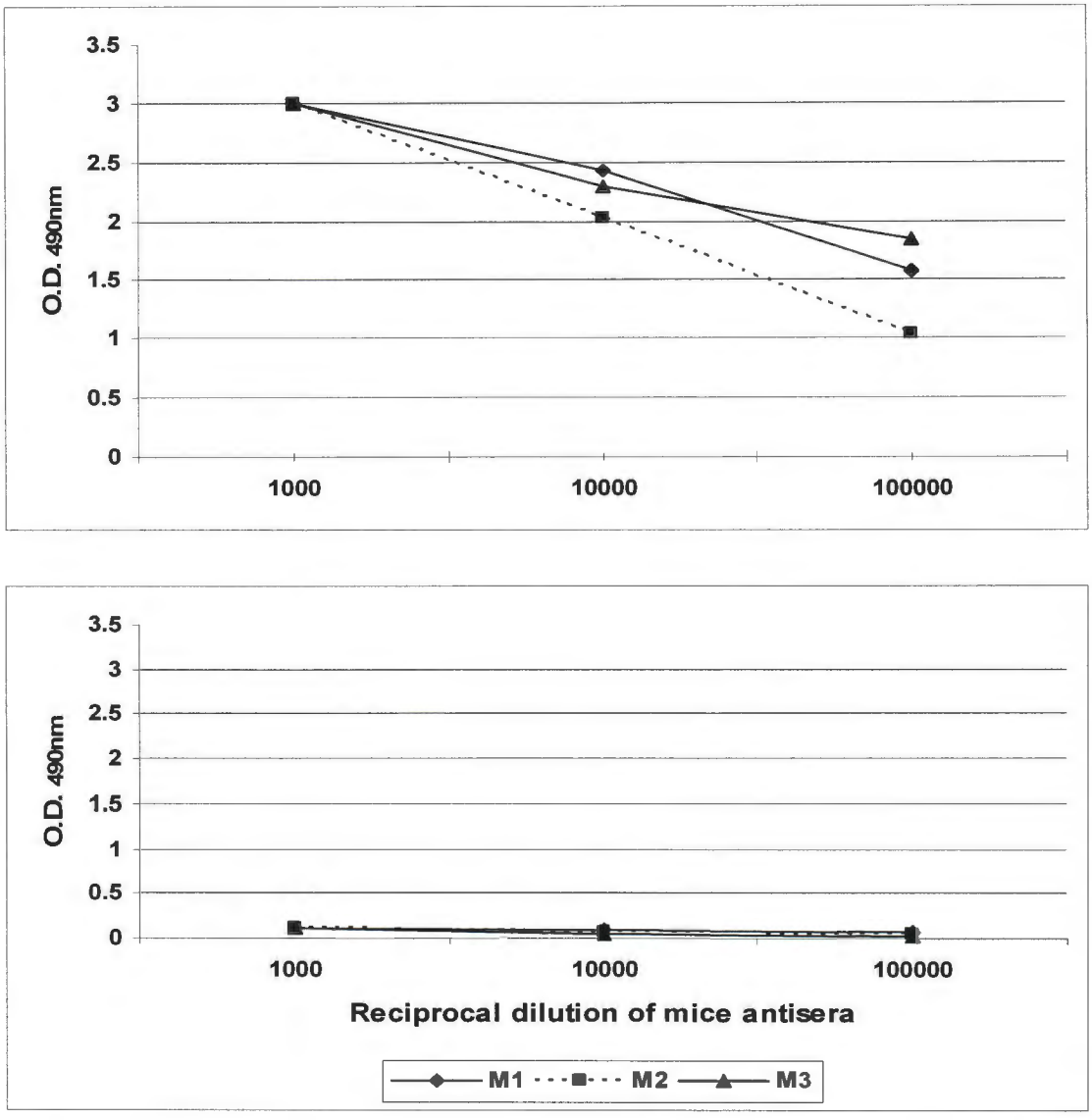

FIGURE 1. Binding curves of mice immune sera to AAb2 and normal pig $F(ab')_2$. Immune sera from mouse immunized with AAb2 were diluted in PBS-T containing 5% NPS and tested for binding to the solid phase AAb2 $F(ab')_2$ (top) and normal pig $F(ab')_2$ (below) by an indirect ELISA. Each data point represents the mean value (SEM) of the OD_{490} obtained from duplicate ELISA results.

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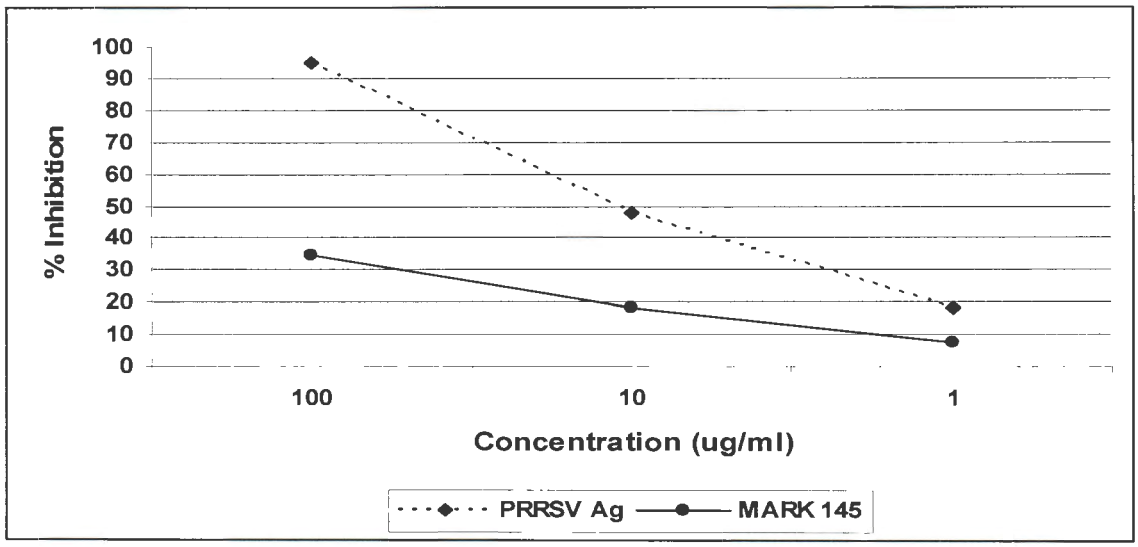


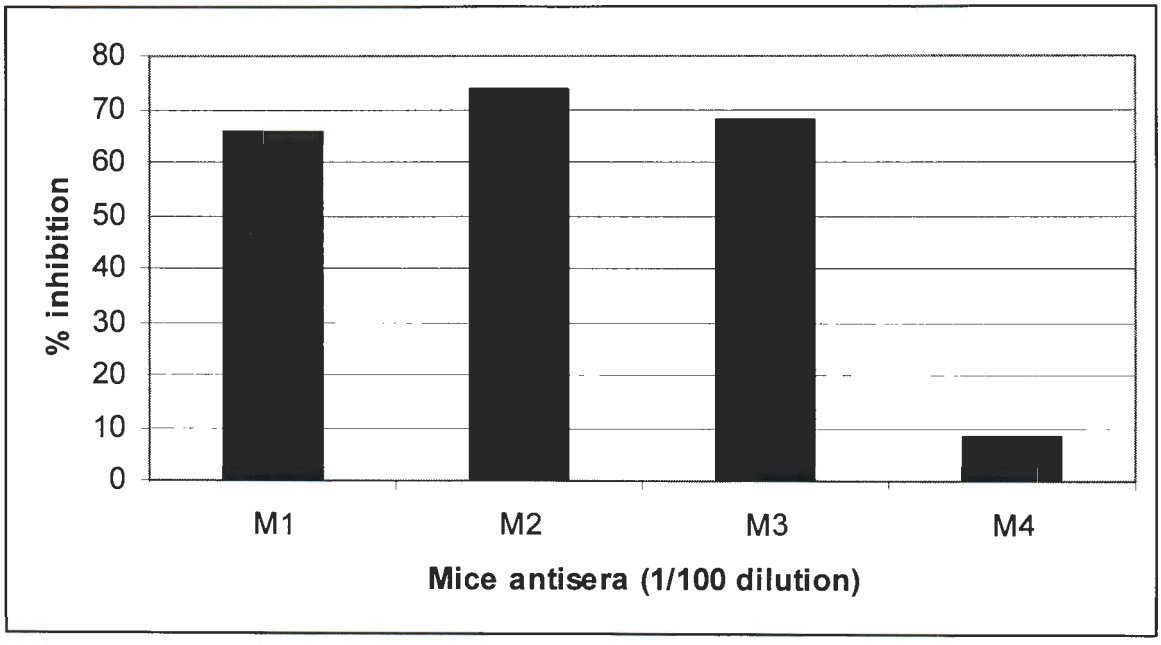
FIGURE 2. Inhibition of the binding of mouse immune serum to AAb2 $F(ab')_2$ fragments. PRRSV antigen along with MARK 145 cell control antigen at different concentrations, were used to inhibit binding of murine serum samples obtained from mice that were immunized with AAb2 immunized to the solid-phase AAb2-$F(ab')_2$. Each point represents the mean (SEM) of the percent inhibition obtained from duplicate ELISA results.

Table 1. Inhibition of binding of mouse immune serum (Ab3) to PRRSV antigen [a] by pig antisera.

Pig no.	Serum Dilution	% Inhibition
P1	1/10	84 [b]
	1/100	34
P2	1/10	31.4
	1/100	28.5
P3	1/10	41.3
	1/100	26.4
P4	1/10	39
	1/100	20.5
P5	1/10	53
	1/100	20.4
P6	1/10	87
	1/100	31.6
P7	1/10	32
	1/100	26.4
P8	1/10	33.5
	1/100	20.9
P9	1/10	35
	1/100	19.9
P10	1/10	57.5
	1/100	41.8
Pre-immune	1/10	6.5
	1/100	1.1

[a] Two hundred nanograms of PRRSV antigen was coated to the solid phase and two different dilutions of individual swine antisera were used to inhibit the binding of mouse immune serum at 1/1000 dilution to antigen.

[b] Each number represents the mean (SEM) of the percent inhibition obtained from duplicate ELISA results.



FGURE 3. Inhibition of AAb2 binding to 25C by Ab3s.

[a] Two hundred nanograms of 25C-F(ab')2 was coated to the solid phase and murine immune sera with AAb2-Fab designated as M1, M2, and M3 diluted in 5% norml pig serum and normal mouse serum (M4), were used to inhibit the binding of AAb2 (100 μg/ml) to 25C-F(ab')2 (2 μg/ml).

[b] Data represent the mean (SEM) of the percent inhibition obtained from duplicate ELISA results.

Table 2. Inhibition of binding of mouse immune serum (Ab3) to AAb2[a] by pig antisera

Pig no.	Serum Dilution	% Inhibition
P1	1/10	64 [b]
	1/100	33.6
P2	1/10	30.5
	1/100	16.2
P3	1/10	34
	1/100	6
P4	1/10	31
	1/100	12.7
P5	1/10	39.5
	1/100	24.5
P6	1/10	60
	1/100	26.4
P7	1/10	30
	1/100	12.5
P8	1/10	36
	1/100	9.7
P9	1/10	36
	1/100	22
P10	1/10	43
	1/100	20.5
Pre-immune	1/10	4.8
	1/100	3.5

[a] Two hundred nanograms of AAb2-F(ab')$_2$ was coated to the solid phase and two different dilutions of individual swine antisera were used to inhibit the binding of mouse immune serum at 1/1000 dilution to AAb2.

[b] Each number represents the mean (SEM) of the percent inhibition obtained from duplicate ELISA results.

CHAPTER 4. GENERAL CONCLUSION

According to the idiotypic network hypothesis proposed by Jerne in 1974, different antigenic determinants within variable domains of immunoglobulins can be recognized and can elicit an immune response in the same individual. These antigenic determinants are known collectively as idiotypes (Ids). According to this original network hypothesis, the Id–anti-Id interactions regulate the immune response of a host to a given antigen. The network hypothesis predicts that within the immune network the universe of external antigens is mimicked by idiotypes expressed by antibodies and T cell receptors. According to the network concept, immunization with a given Ag will generate the production of antibodies against this Ag termed Ab1. This Ab1 can generate a series of anti-Id antibodies against Ab1 termed Ab2. Some of these Ab2 molecules can effectively mimic the three-dimensional structures of external antigens. These particular anti-Ids called Ab2β, which fit into the paratopes of Ab1, can induce specific immune responses similar to responses induced by nominal Ag. Immunization with Ab2β can lead to the generation of anti-anti-Id antibodies (Ab3) that may recognize the corresponding original Ag identified by the Ab1. Because of this Ab1-like reactivity, the Ab3 is also called Ab1' to indicate that it might differ in its other idiotopes from Ab1.

In the first study, we described the generation and characterization of a monoclonal anti-Id, designated as M8G, directed to an Id on monoclonal antibody, 25C. 25C recognized an epitope referred to as glycoprotein 5 (GP5) on the PRRSV. Based on the following results we concluded that anti-Id M8G recognized an Id either within or near the antigen combining

sites of MAb1-25C and possessed common Id to antibodies that are formed to GP5 in unrelated species: (i) Id-anti-Id interaction could be inhibited by PRRSV antigen, and (ii) Anti-Id inhibited the interaction between MAb1 and PRRSV antigen. According to the classification of anti-idiotypic antibodies, the anti-Id M8G has the characteristics of an internal image anti-Id.

To further study of Id network in PRRSV infection, monoclonal anti-Id (M8G) was used to induce an anti-anti-idiotypic antibodies (Ab3) response in murine model. Based on the following results we concluded that monoclonal anti-Id produced the anti-anti-Id that possessed Ab1-like (Ab1') properties with specificity for the PRRSV antigen in murine model: (i) the binding of anti-anti-Id to anti-Id was inhibited by PRRSV antigen, (ii) anti-anti-Id antibody competed with Ab1 binding to PRRSV antigen, and (iii) Id-Anti-Id interaction was inhibited by Ab3. Investigations using a murine model system are in progress to determine whether the anti-Id M8G can modulate the immune response to PRRSV.

To further investigation of Id-anti-Id network in PRRSV infection, the second study was conducted using swine auto anti-idiotypic antibody (AAb2). We have previously identified pig auto anti-idiotypic antibody specific for GP5 of PRRSV, and characterized AAb2 as an internal image anti-Id (Ab2β). In this study, BALB/c mice were immunized with AAb2 and the immune sera (Ab3) were tested for the presence of anti-GP5 antibodies and the expression of the Id of Ab1 specific for GP5 of PRRSV.

We concluded that immunization of mice with AAb2 led to the generation of an anti-GP5 antibody response with epitope specificity similar to that of Ab1 (Ab1-like or Ab1' response)

in murine model for the following reasons: (i) Id-anti-Id interaction was inhibited by Ab3, (ii) the binding of anti-anti-Id to AAb2 was inhibited with PRRSV antigen, (iii) pig antisera to PRRSV inhibited the interaction between Ab3 and PRRSV antigen, and (iv) Ab3-AAb2 interaction was inhibited by pig antisera. The finding that the binding of AAb2 to MAb1 (25C) was inhibited by Ab3, and pig antisera (Ab1) inhibited interaction between Ab3 and anti-Id, show that auto-anti-Id produced the anti-anti-Id which recognized the idiotypes that were shared by mouse and pig anti-PRRSV antibodies.

Further studies on anti-PRRSV antibody and anti-idiotypic antibody responses and their relationship with cellular immune responses would be necessary to understand the immune responses against PRRSV and to develop a strategy to control PRRSV antigen.

ACKNOWLEDGMENTS

I would like to thank my major professor, Dr. En-Min Zhou, who has been an excellent mentor. He has provided me with invaluable advice and guidance throughout my entire research project. Dr. Jeffery Zimmermann has many thanks as well for his guidance, patience and encouragement throughout this project.

For great advice on editing the content of my thesis and serving on my committee, I would like to thank Dr. Michael Wannemuehler.

I am also grateful to the past and present members of Dr. Zhou's laboratory. Dr. Zhengjun Jiang, Dana Skibbe, and Patrick Emge made significant contributions to the scientific merit of my thesis work, all of which is greatly appreciated. I would like to thank Dr. Kenneth Platt for providing monoclonal 25C. I also wish to express my deep appreciation to all Serology Section members for their patience and always being ready to help. Finally, I would like to thank Dr. Gary Osweiler for supporting me during my graduate study.